THE UNIVERSITY OF ALBERTA

SOME ASPECTS OF THE PHYSIOLOGY OF EARLINESS IN VEGETABLE CROPS

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ABSTRACT

A considerable amount of work has been done on the physiology of flowering but little information is available concerning the physiology of vegetative earliness as distinct from physiology of early flowering. Although early maturity has been influenced by physiological or genetic manipulations, little attention has been paid to what physiological process or processes in the plant are responsible for early maturity. Hence attempts were made to determine what physiological factors, if any, had any correlation with earliness.

With each of the four vegetable crops used in these studies a correlation was found at some stage of growth between phosphorus content in leaf tissue and earliness. In tomatoes and lettuce negative correlations were obtained between phosphorus content of leaf tissue and days to maturity. In cabbage and radish the correlation was found to be positive. The correlations were highest at earlier stages of plant growth and differences in phosphorus level of varieties were also greater at earlier stages of plant growth. In general there was a decrease in phosphorus content of leaf tissue as the age of the plant increased.

Positive correlations were found between chlorophyll (a + b) content of leaf tissue and days to maturity in three crops, tomato, lettuce and cabbage. In contrast to the differences in phosphorus level, which were greater at earlier stages, differences in chlorophyll levels were more apparent as the plants advanced in age.

In tomato varieties there was a negative correlation between malic acid content of fruit juice and days to maturity of the varieties from green mature stage of fruit to red ripe stage. At the mature green stage the malic acid content was higher than at early or late ripe stages in all varieties tested.

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INTRODUCTION

Early maturity is a very important factor in the production and marketing of horticultural crops. In regions of long growing season produce supplied to the market early in the season usually brings a premium price. Earlier maturity also makes possible an extension of the marketing period in these regions. A longer marketing period generally results in greater economic return than a short one. In the Great Plains Region of North America, where shortness of the growing season is recognised as an important limiting factor in the production of some crops, earliness is of even greater importance - as only those varieties, which mature within this short period, can be grown successfully.

Earlier maturity has been achieved to some extent by certain physiological manipulations and cultural techniques, examples of the former are application of plant growth regulators such as CCC and a very recent material not yet named but identified as NIA 8198 (33). In the opinion of many, however, the most important method of obtaining earlier production is the breeding of early maturing varieties. The breeder must grow and observe large populations of several segregating generations to achieve his objective. Such a procedure involves a great deal of time and expenditure. If some indication of earliness could be obtained at an early stage of growth, plants would not have to be grown to maturity. As a consequence, plant breeders could eliminate the undesirable bulk of plants in early stages

of testing and thus reduce population size to a considerable extent.

If a correlation could be established between plant constituents and earliness, breeding for earliness might be approached on the basis of genes responsible for plant constituents rather than those responsible for early maturity.

An investigation was undertaken, therefore, to determine what correlations, if any, existed among some of the plant constituents and earliness. Since the level of phosphorus available to the plant is related to maturity the possible correlation between phosphorus content of the plant tissue and earliness was studied. This project is presented in Part I.

Studies were also undertaken to determine whether total chlorophyll (a + b) content of the leaf tissue had any correlation with earliness. This problem is dealt with in Part II.

It has been reported that some of the organic acids in tomato fruit juice have some correlation with early ripening of tomato fruits (22). Hence studies of the relationship of organic acids in tomato fruit juice to earliness were attempted. These studies are outlined in Part III.

The crops involved in the investigations included tomato, lettuce, cabbage and radish.

REVIEW OF LITERATURE

Introduction

The amount of literature presently available on the physiology of flowering (25, 40) provides considerable insight into the understanding of the various factors responsible for the flowering of plants. The manipulation of some of these factors such as light, temperature, and certain growth regulators, have accelerated flowering in many species.

In contrast, the amount of information available on the physiology of vegetative earliness, as distinct from earliness of flowering, is very meager. Although some manipulations have resulted in hastening vegetative maturity apparently no attempts have been made to determine what physiological factors within the plant are responsible for it.

A. The Importance of Phosphorus in Plant Growth

Farmers and agricultural workers recognized the important role of phosphorus in crop production and its relation to earliness, long before its function in growth and metabolism was discovered. Bone, the first important source of phosphorus fertilizer, was used in Great Britain as early as 1774. Over a hundred years ago England was importing such large quantities of bone from the continent of Europe that Liebig, the noted German chemist, wrote with some alarm, "England is robbing all other countries of the condition of their fertility."

In recent years great advances have been made in understanding the physiological functions of phosphorus. It is involved in conservation and transfer of energy in the metabolic reactions of living cells (27, 28). Phosphorus is present in plants as the inorganic phosphate radical and is one of the component elements of several organic compounds such as the phospholipids, phytin, the phosphorylated sugars, the nucleoproteins, the phosphoproteins and nucleic acid (1).

1. The Role of Phosphorus in Reference to Maturity

It is a well known fact that application of phosphatic fertilizers hastens maturity. Sommer (43) explained that the application of large quantities of phosphorus had an indirect effect on the hastening of maturity. There was greater growth with increased application of phosphorus and owing to its more rapid growth the plant absorbed certain other ions more readily. This increased uptake of some other elements, perhaps nigrogen, resulted in such nutrients becoming limiting factors, which in turn caused early maturity. The reverse effect, for example, an increase in nitrogen supply resulted in a decrease in leaf phosphorus (7, 41).

2. The Selective Absorption of Nutrients by Different Species and Varieties

Plants of different types differ widely in both nutrient requirements and the ability to absorb the various elements from a common medium. Plants, by means of mechanisms not thoroughly explained have the capacity of selective absorption

and this selectivity varies both qualitatively and quantitatively with the species (41). Selective absorption was reported by Newton (30) as early as 1928.

Foote and Howell (15) compared the phosphorus uptake of two soybean varieties. Increasing phosphorus supply stimulated phosphorus uptake more in the phosphorus sensitive variety Lincoln than in the phosphorus tolerant variety Chief.

Under low phosphorus conditions in the cotton plant there were rather consistent decreases in the percentages of calcium, magnesium, potassium and sulfur. Iron percentages were not influenced (13).

3. The Effect of Phosphorus Deficiency in Nitrogen Metabolism

When supplies of phosphorus were low in cotton plants the concentrations of nitrate and protein nitrogen were both depressed, whereas soluble-organic nitrogen was increased, indicating a blocking of protein synthesis (13). Eaton (10) observed similar results in soybean plants. The accumulation of carbohydrates in the middle phase of phosphorus deficiency is generally considered to be due to a cessation of protein synthesis. Pirson (34) did not consider accumulation of carbohydrates to be exclusively due to cessation of protein synthesis. He believes such an accumulation to be partly due to stunting of shoots causing a relatively higher leaf/stem ratio. Pirson also noted a reduction in total sugars and protein due to phosphorus deficiency in oats. Hence phosphorus deficiency causes disturbances in nitrogen metabolism.

Lowering of the shoot/root ratio is considered a general characteristic of phosphorus deficiency. It is suggested that such a lowering of the shoot/root ratio, associated with phosphorus deficiency, is a result of decrease in the auxin level of the whole plant.

4. Translocation of Phosphorus in Plants

Unlike certain inorganic nutrients such as calcium, phosphate has long been observed to be a mobile nutrient within the plant. Phosphate does not remain fixed in the cell or tissue in which it was originally deposited but is able to move from one site to another. The requirement for phosphate is highest in the youngest cells or those characterized by a high rate of metabolic activity. When the external supply of phosphorus becomes limiting, there occurs a redistribution of the phosphorus already contained in the plant; phosphate is withdrawn from the older, metabolically less active cells and moves into the younger, metabolically more active cells. Hence circulation of phosphorus within the plant is in accordance with the "Physiological priority" pattern (1).

Plants such as cereals, which are characterized by a determinate type of growth, do not require a continuous supply of phosphorus during their life cycle. The phosphorus absorbed in the early stages of growth and deposited in vegetative tissue is redirected to the seed at the stage of its active formation. Gericke (16) could grow a normal wheat crop in solution culture by supplying phosphate to the plants only during the first

four weeks of their growth. Howell (19) also found that when soybean plants were grown on a high phosphorus level for the first eight weeks, phosphate supply could later be eliminated without any affect on yield.

In plants such as tomato, which have an indeterminate type of growth, a somewhat different pattern of phosphate distribution was observed. Arnon and Hoagland (2) grew tomato plants for five weeks in complete nutrient solutions amply supplied with phosphate. Half of the plants were then removed to minus phosphate cultures where fruits were formed at the expense of the phosphate absorbed by the vegetative tissue during the first five weeks. Vegetative growth of the fruiting plants was restricted. The remaining plants, deflowered and grown in the minus phosphate cultures made approximately twice the vegetative growth of those allowed to fruit.

5. <u>The Relationship of Phosphorus Level in Plant Tissue to Nutrient Supply and Physiological Age</u>

The phosphorus in plants occurs in greater amounts in seeds and fruits than in the rest of the plant, and the total content in tissues depends upon the amount in the soil (17).

The supply of phosphate in the soil affects to a great extent the amount of phosphorus in leaf tissue. Roberts and Kenworthy (38) grew strawberries in different strengths of Hoagland's solution, ranging from one tenth to full strength. Total growth was not affected but the tissue concentration of K, P, B and Cu increased as the supply of these nutrients in

the solution increased. Ca, Fe and Mn tended to decrease with increasing supply in the nutrient solution and Mg was not affected.

Plants receiving sufficent phosphorus showed a steady increase in tissue phosphorus from the lower leafy parts of the plant to the young growth at the top, but when phosphorus supply was limited they had approximately the same percent phosphorus in all regions of the stem and leaves (12).

Another important factor affecting the mineral composition is physiological age of tissue. According to Smith (41) nitrogen, phosphorus and potassium decreased with increasing age of leaf tissue in most of the vegetable crops and calcium increased.

Phosphorus is present in high concentration in young tissue and is diluted as tissue enlarges. Carbohydrate accumulation also dilutes phosphorus concentration (41).

6. Other Factors Affecting Phosphorus Content of Plant Tissue

Soil moisture supply and irrigation usually cause appreciable modification of tissue composition (41). Wilson (50) measured the concentration of acid soluble phosphorus compounds in one month old *Trifolium subterraneum* plants at several stages of soil moisture depletion and after 24 hours recovery from moisture stress. The concentration of various phosphorus compounds decreased in plants whose relative turgidity was 50 - 70%. In severely wilted plants (relative turgidity 20 - 45%), the concentration of most phosphorus compounds decreased more than 50% compared to fully turgid

plants. When the plants were irrigated there was a rapid increase in phosphorus content in them.

The carbon dioxide concentration in the atmosphere affected the uptake of phosphorus by chrysanthemums, geraniums and cucumbers as reported recently by McEvoy (29). Over the range of 500 to 1500 ppm there was a progressive increase in the rate of uptake of radiophosphorus in the three plant species with increasing carbon dioxide concentration.

Availability of other nutrients had antagonistic (7) or synergistic (13) effects on phosphorus levels of plants. Increasing nitrogen and boron level decreased phosphorus content in citrus leaf tissue (41).

The application of growth regulators has been reported to affect phosphorus content of plant tissue. Cathey found that total ^{32}P in CCC treated barley seedlings was three to four times as large as in the nontreated control plants (8). In another study Linck (24) applied 1 ppm of gibberellic acid to roots of "Black Valentine" variety of *Phaseolus vulgaris* L. Plants treated with gibberellic acid under both rapid and restricted transpiration conditions absorbed more ^{32}P than non-treated ones. After 28 hours significantly more amounts were found in actively growing tissues of the plant.

Humphries (20) found higher amounts of phosphorus in hypocotyles of dwarf beans due to application of indoleacetic acid and naphthaleneacetic acid. Inorganic phosphorus uptake was accelerated about 200% shortly after treatment of cotton and coleus plants with 2,4-Dithiobiuret (36) In another

study Ormrod (32) observed an increase in organic phosphorus and corresponding decrease in inorganic phosphorus after spraying plants with 2,4-D and gibberellic acid. Smith (42) also noted an effect on phosphorus uptake as a result of 2,4-D treatment.

Fedorov (14) found that IAA and 2,4-D application of apple and golden currant cuttings inhibited absorption of phosphorus.

Other environmental factors also affected the mineral content in plant tissue. Soil pH and temperatures influenced to a great extent the mineral uptake by plants (41).

B. The Relation of Chlorophyll to Earliness

The production of chlorophyll is a process dependent on many factors. It is influenced by external, nutritional, and genetic factors. Some of these factors which might have some possible relationship with earliness are discussed briefly here.

1. Nutritional Factors

A deficiency in some inorganic nutrient may limit photosynthesis through depression of the chlorophyll content of leaves. Deficiencies of sulfur, nitrogen, iron and magnesium may lead to chlorophyll limitation (23).

A deficiency of nitrogen, an essential component of the chlorophyll molecule, leads to a reduction in the chlorophyll content resulting in a yellowing of leaves.

Pirson (35) reported that chlorophyll content in leaf tissue increased with phosphorus deficiency. Similar results

were reported by Eaton (11, 12). He observed that a major symptom of phosphorus deficiency in the early stages of development of soybean plants was a deeper green colour of the leaves. Similar results were reported in mustard plants.

In is generally recognized that nutritional factors influence the production of chlorophyll (11, 12, 23, 35). Certain nutrients are also known to influence earliness (7, 41, 43). Hence a relationship between chlorophyll and earliness may exist.

2. Genetic Factors

Most chlorophyll mutants are simple Mendelian recessives, indicating that mutations are due to the action of single genes. In maize 13 genes are known which independently produce albino seedlings. Several genes are known which result in pale green seedlings where the amount of chlorophyll is reduced at certain stages of development. In one albino strain of corn it was found that light caused an excessive rate of destruction of chlorophyll. Several corn mutants were found to be partially defective in the rate at which they made protochlorophyll, but they possessed the mechanism for the continued production and conservation of chlorophyll. One of the barley mutants, chlorine 2, was found to possess a normal content of chlorophyll a but was devoid of chlorophyll b (18).

Another group of inheritable factors which may influence chloroplast development may have cytoplasmic inheritance. Some 20 cases of cytoplasmic inheritance have been discussed (18).

It has been reported that production of chlorophyll is controlled by genetic factors (18). In addition it is well established that earliness of plants is influenced by genetic factors. It might be suggested, therefore, that some relationship (linkage) may exist between those genes influencing earliness and those influencing chlorophyll production.

3. Some Effects of Growth Regulators on Chlorophyll Content

The application of growth regulators influence the chlorophyll content of leaf tissue. According to Wittwer (51) the new growth after gibberellin treatment was often paler green or chlorotic. This could be partially offset by maintaining high fertility level, particularly nitrogen.

Norris (31) reported that application of CCC increased chlorophyll content in leaf tissue.

Growth regulators have been reported to hasten maturity and to influence chlorophyll production. There may be some relationship between chlorophyll production and early maturity.

4. The Relationship of Chlorophyll Content of Leaf Tissue to Dry Matter Content of Plants

Bray (5) has shown a highly significant correlation between the dry weight of the above-ground crop of several annual herbaceous stands and the concentration of chlorophyll per unit area of land surface. The relationship shown was for the stands at their maximum total yield. Hence Bray demonstrated the feasibility of using total chlorophyll content as an index of net annual productivity of undisturbed herbaceous stands.

Brougham (6) obtained similar results when some pasture and crop plants were tested. The species tested were red clover, white clover, perennial ryegrass, maize, kale, cocksfoot and water cress. There was a highly significant correlation of +0.912 between maximum growth rate of different species and amount of chlorophyll per unit area of land.

C. Some Relationships Between Organic Acids and Earliness

Organic acids play a very important role in the physiological processes of plants. They are the first products of photosynthesis and help in the building of carbohydrates, fats and proteins. They also effect oxidative reduction of sugars and are involved in respiration. The dicarboxylic and tricarboxylic acids are the main acids present in the higher species of plants. Among the most commonly found are oxalic, malic, tartaric and citric acids.

Koch (22) has found a positive correlation between the geographical location of the carboxylic acids and temperature. It has been observed that plants which contain malic acid, use it for respiration at 12° C. Tartaric acid is utilized at 20° C and citric acid at 30° C. Those plants that contain malic acid are habitants of the temperate zone. Plants containing mainly citric acid are the habitants of Mediterranean and subtropical regions.

Koch (22) tested the quantitative changes of malic acid and citric acid content in tomato fruits of various varieties, ranging in maturity period from early to very late types. The

determinations were made at seven different stages of fruit development. He observed that both malic acid and citric acid reached a peak in the final fruit development and green ripening stages. In earlier ripening varieties the malic acid was present until the dead ripe stage whereas in late ripening varieties there was no malic acid present after the green ripening stage. On the basis of malic acid test in 18 varieties a negative correlation between quantity of malic acid in tomato fruit juice and days from flowering to ripening of fruits in different varieties was significant at the 1% level.

An increase in organic acids has been noted in ripening fruits. Wyman (52) reported an increase from 35 to 65% of malic acid from green to ripe pulp of banana fruits. Robertson (39) found a rapid increase in total organic acids especially malic acid in developing apple fruits.

PART ONE

THE RELATIONSHIP BETWEEN EARLINESS AND PHOSPHORUS CONTENT OF LEAF TISSUE IN FOUR SPECIES OF VEGETABLE CROPS

Four vegetable crops were selected for these studies; one so-called fruit crop; i.e., tomato, two leafy vegetable crops - lettuce and cabbage and one root crop - radish. Lettuce, cabbage and radish were grown in the field and the greenhouse. Tomatoes were grown in the greenhouse only. Varieties of varying maturity periods from very early to late were selected from each crop. The random selection of varieties was partly based on availability of varieties in certain maturity groups.

A. FIELD EXPERIMENTS

Field experiments were conducted at Parkland Field Laboratory during the summer 1965. The official classification of the soil is Malmo Silt Loam (4) which is an eluviated black soil, developed on Lacustrine material. On the basis of arable quality it has been classed as a good to very good soil.

The average frost free period in Edmonton is about 100 days. The mean annual precipitation is 16 to 18 inches. The mean summer temperature, May to September, is 56° F. July is the warmest month averaging 61.5° F.

Soil samples were taken from the plots selected for growing various crops and analysed for available nutrients. The available nitrogen was 40 - 45 lbs per acre. The available phosphorus in the soil was 40 - 50 lbs per acre. The available

potassium was very high, 550 to 600 lbs per acre. The pH ranged from 5.5 to 5.9 and the soil contained a medium amount of organic matter.

MATERIALS AND METHODS

I. Lactuca sativa var. capitata L.

Six varieties of lettuce varying in maturity period from early to late types were selected for these studies. The names of the varieties and sources of seed are listed below:

Sample Number	Variety	Source of Seed
1	New York 12	Steele Briggs Seeds, Edmonton
2	Pennlake	Steele Briggs Seeds, Edmonton
3	Great Lakes 659	A.E. McKenzie Co. Ltd., Seedsmen, Edmonton
4	Premier Great Lakes	Steele Briggs Seeds, Edmonton
5	Francisco	U.S. Department of Agriculture
6	Alaska	A.E. McKenzie Co. Ltd., Seedsmen, Edmonton

The seeds were sown in a greenhouse on April 15, 1965. The seedling medium placed in flats consisted of a 1:1 sand peat mixture. Row spacing was three inches. After germination the plants were pricked out and transplanted into a 3:2:1 (soil : sand : peat) mixture in three inch veneer bands. Each plant was watered with 100 ml of a starter solution prepared by dissolving 1 oz of 10-52-17 per gallon of water. The starter solution application was repeated every ten days prior to field setting in Parkland Field Laboratory.

One week before field setting the plants were transferred to cold frames for hardening. After the hardening treatment the plants were set in the field and 500 ml per plant, of the afore mentioned starter solution were applied.

In this preliminary investigation no regular experimental design with replications was used. However, the varieties were planted in random order. One row, ten metres long, with 25 plants was used for each variety. The planting distance was one metre between rows and 40 centimeters between plants.

Two weeks after field setting 1.40 kg of 27-14-0 fertilizer were applied to the whole area of 60 square metres. All plants were supplied with an equal amount of fertilizer. The fertilizer was spread around the plant and raked into the soil.

Sampling Plant Leaf Tissue

Leaf tissue samples were taken nine weeks after germination by the method of Ulrich and Smith (41, 46). The youngest fully matured leaf was selected for this purpose and the sample included the whole leaf, both leaf blade and petiole. Single leaves were taken randomly from fifteen plants of each variety. The 15-leaf samples were dried at $55 \pm 2^{\circ}$ C in a forced draft oven and ground to a fine powder. The powder was homogenized by thorough mixing and two samples from each variety were taken for phosphorus determinations. The average of these two determinations was taken as the phosphorus content of the variety.

Dry Matter Determination

The percentage dry matter in different varieties was determined by the method of Ward (48).

Phosphorus Determinations

The powdered dry leaf tissue samples were held in an oven at 60° C for two hours and cooled in a dessicator before weighing. The samples were weighed in a porcelain crucible on an analytical balance soon after being removed from the dessicator. Total phosphorus determinations were made by the Chapman and Johnson method (9, 21) as modified by C.F. Bentley for use in the Agricultural Soil and Feed Testing Laboratory, University of Alberta.

The four main steps in the determination of phosphorus were:

(1) Ignition with Magnesium Nitrate.

(2) Preparation of Ammonium molybdate-ammonium vanadate reagent.

(3) Spectrophotometric reading.

(4) Preparation of Phosphate standard.

Details of each procedure were as follows:

1. Ignition with Magnesium Nitrate

5 ml of 50% magnesium nitrate solution were added to each sample in the crucible and a small amount of magnesium oxide added to each. The liquid was evaporated at low heat on a hot plate, the heat was then increased until the reaction was complete and the sample was completely dried. The dried sample was transferred to a muffle furnace, maintained at 800° F, for half an hour for ashing.

After cooling, the ash was dissolved in 15 ml of concentrated hydrochloric acid and filtered through a Whatman No. 1 filter paper into a 200 ml volumetric flask. The filter paper was washed with repeated aliquots of distilled water, volume made up to the mark and the solution mixed thoroughly.

2. Preparation of Ammonium Molybdate-ammonium Vanadate Reagent

The reagent was prepared as follows. 22.5 gm of ammonium molybdate were dissolved in 400 ml of water. 1.25 gm of ammonium vanadate were dissolved separately in 300 ml of boiling water. The ammonium vanadate solution was added to the ammonium molybdate and cooled to room temperature. To this was added 250 ml of concentrated nitric acid. The mixture was then made up to one litre with addition of distilled water.

3. Spectrophotometric Reading

10 ml aliquots from the 200 ml volumetric flasks were transferred to a 50 ml volumetric flask. To each of these 10 ml aliquots 10 ml of ammonium molybdate-ammonium vanadate reagent was added and the volume was made up to 50 ml with distilled water. The contents were mixed thoroughly and allowed to stand for two hours for the development of color.

Absorbance was measured with a Universal Spectrophotometer at a wave length of 415 mμ against a blank standard made by diluting 10 ml of reagent to 50 ml with distilled water.

Two readings were taken from each sample and the average of these used for phosphorus estimation.

4. Preparation of Phosphate Standard

A phosphate standard was made by dissolving 0.2195 gm of potassium phosphate in distilled water and diluting to two litres. The standard solution was 25 ppm phosphorus.

Aliquots of the standard solution were transferred to 50 ml volumetric flasks. 10 ml of ammonium molybdate-ammonium vanadate reagent were added to each and made up to 50 ml. The absorbance was measured in the same way as for plant tissue samples. A standard curve was established with the absorbance readings from known concentrations.

Absorbance readings of the plant leaf tissue samples were compared with the standard curve and the phosphorus content of individual samples thereby estimated. From these estimations the percentage phosphorus in dried leaf tissue was calculated.

II. *Brassica oleracea* var. *capitata* L.

Eight cabbage varieties of varying maturity periods were selected for this investigation. The names of the varieties and sources of seed are given below.

Sample Number	Variety	Source of Seed
1	First Acre	A.E. McKenzie Co. Ltd., Seedsmen, Edmonton
2	Dwarf Morden	Horticultural Station, Brooks, Alberta
3	Early Round Head	A.E. McKenzie Co. Ltd., Seedsmen, Edmonton
4	Early Golden Acre	A.E. McKenzie Co. Ltd., Seedsmen, Edmonton
5	Copenhagen Market	Steele Briggs Seeds, Edmonton

Sample Number	Variety	Source of Seed
6	Glory of Enkhuizen	Steele Briggs Seeds, Edmonton
7	Danish Ballhead	Robertson's Seed and Feed Ltd., South Edmonton
8	Penn State Ballhead	Dominion Seed House, Georgetown, Ontario

Cabbage plants were started in the same manner as the lettuce plants. In this preliminary study no regular experimental design was used. The varieties were planted in random order with one 10 metre row for each variety. The planting distance was one metre between rows and 50 cm between plants, with 20 plants per row.

Two weeks after field setting 2.50 kg of 27-14-0 fertilizer mixture were applied to the area involved in the test.

The leaf tissue samples were taken ten weeks after germination by the method previously discussed. Drying and estimation of total phosphorus was also done in the same manner.

III. *Raphanus sativus* L.

Seven radish varieties were selected for these studies. The seven varieties and their sources are listed below.

Sample Number	Variety	Source of Seed
1	Cavalier	Steele Briggs Seed Co., Edmonton
2	Champion	Steele Briggs Seed Co., Edmonton
3	Forcing Scarlet Globe	Steele Briggs Seed Co., Edmonton
4	Sparkler	Steele Briggs Seed Co., Edmonton
5	Long White Icicle	Steele Briggs Seed Co., Edmonton
6	Chinese Rose	Steele Briggs Seed Co., Edmonton
7	Long Black Spanish	Steele Briggs Seed Co., Edmonton

The seeds were sown in plots at the Field Laboratory on May 18, 1965. Rows were one metre apart. One row five metres long was used for each variety and the order of varieties was randomized. After germination the plants were thinned to about 5 centimeters between plants. Ten days after germination 1.50 kg of 16-20-0 fertilizer were applied to the whole area as a side dressing.

The leaf tissue sampling was done by the method of Ulrich (46). The drying, grinding and phosphorus determinations were as described for the lettuce crop.

In addition to sampling for phosphorus and dry matter determinations records of maturity period for the different varieties of lettuce, cabbage and radish were obtained to determine the average maturity period of the varieties.

The average maturity period of the lettuce crop was determined by the number of days from germination to harvest of 60% of the heads. Generally not more than 60 - 70% of the heads attain normal size and shape due to great variability in maturity of the crop.

The average maturity period of cabbage was measured by recording the number of days from germination to harvest of 70% of the heads since maturity in cabbage was more uniform than in lettuce.

RESULTS

I. Lettuce

There were no significant differences in average dry matter content among varieties of different maturity period.

The correlation of % phosphorus in leaf tissue with days to maturity was -0.75. This correlation was not significant. The phosphorus content of leaf tissue was higher in earlier varieties than in later ones. The results are presented in Table I and Figure I.

II. Cabbage

As will be seen in Table II, the later varieties had a higher percentage dry weight than earlier varieties. The later varieties also had more phosphorus in leaf tissue than did earlier varieties. The variety Penn State Ballhead had about 24% more phosphorus in leaf tissue than First Acre. The correlation of +0.894 between phosphorus content of leaf tissue and days to maturity was significant at the 1% level. The levels of phosphorus in leaf tissue are summarized in Figure II. The regression coefficient of % phosphorus on days to maturity was +0.00276 (Figure III).

III. Radish

There were no differences in dry matter content among varieties. However, later varieties had more phosphorus content of leaf tissue than did the earlier ones. The positive correlation between percentage phosphorus and days to maturity was significant at the 5% level. The results are presented in Table III and Figure IV. The regression of % phosphorus on days to maturity is shown in Figure V and the slope of the line is +0.00745.

TABLE I

Days to maturity of six varieties of Lactuca sativa var. capitata L., average % dry matter and % phosphorus content in dry leaf tissue at nine weeks after germination

Sample Number	Variety	Days from Germination to 60% Marketable Heads	% Dry Matter	% Phosphorus
1	New York 12	77	6.343	0.51
2	Pennlake	82	6.669	0.44
3	Great Lakes 659	83	7.202	0.45
4	Premier Great Lakes	86	7.712	0.34
5	Francisco	92	7.276	0.38
6	Alaska	95	6.355	0.36
Correlation between % phorphorus in leaf tissue and days to maturity				-0.75 (N.S.)

FIGURE I

Percentage Phosphorus in leaf tissue of six varieties of field grown *Lactuca sativa* var. *capitata* nine weeks after germination.

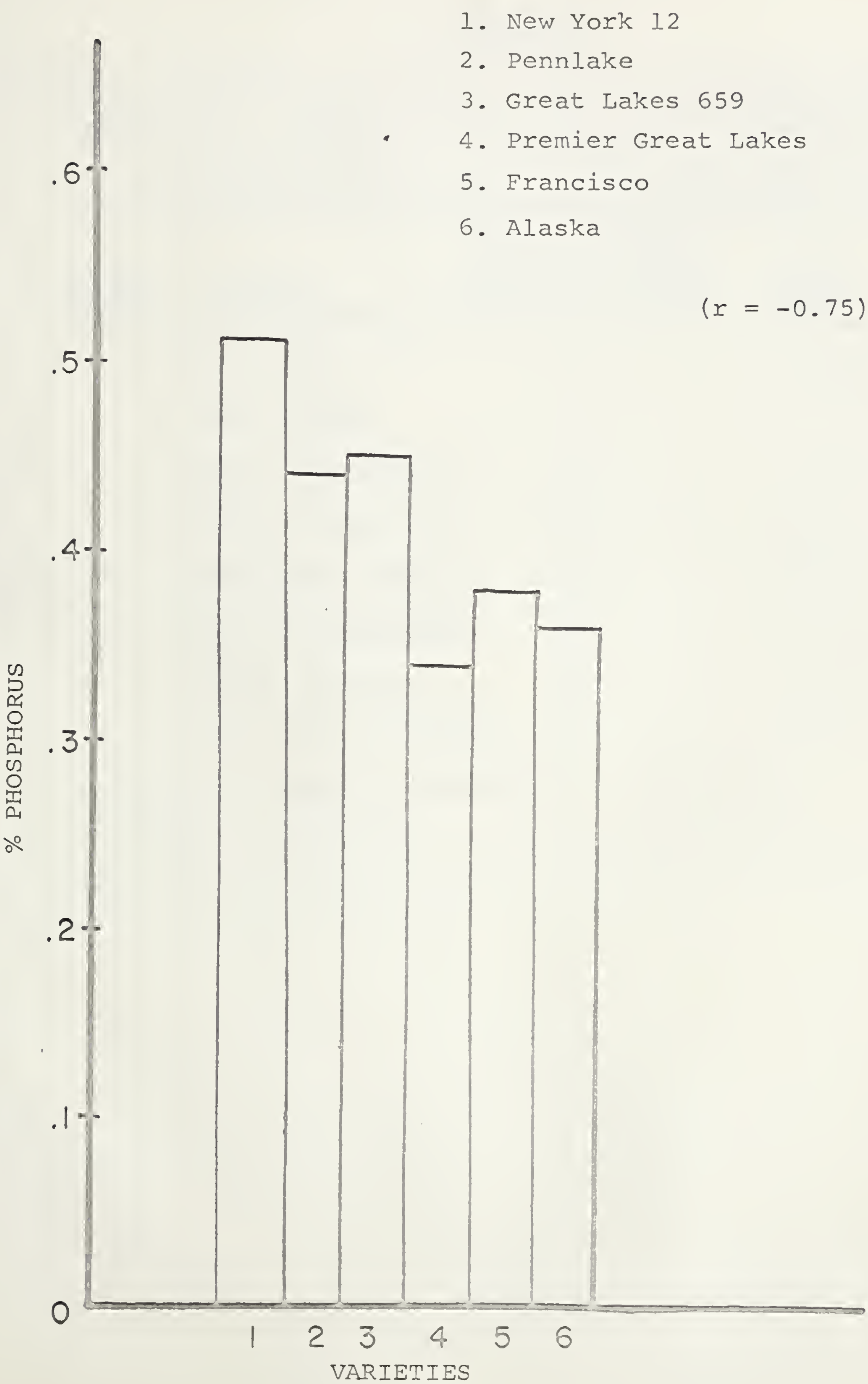


TABLE II

Days to maturity of eight varieties of *Brassica oleracea* var. *capitata* L., average % dry matter and % phosphorus content in dry leaf tissue at ten weeks after germination

Sample Number	Variety	Days from Germination to 70% Marketable Heads	% Dry Matter	% Phosphorus
1	First Acre	96	9.190	0.50
2	Dwarf Morden	98	9.333	0.43
3	Early Round Head	98	9.797	0.51
4	Early Golden Acre	111	10.623	0.52
5	Copenhagen Market	115	11.568	0.53
6	Glory of Enkhuizen	135	11.299	0.61
7	Danish Ballhead	148	11.280	0.62
8	Penn State Ballhead	152	11.322	0.62
Correlation between % phosphorus in leaf tissue and days to maturity				+0.894**

**Significant at 1% level.

FIGURE II

Percentage Phosphorus in leaf tissue of eight varieties of field grown <u>Brassica</u> <u>oleracea</u> var. <u>capitata</u>, ten weeks after germination.

1. First Acre
2. Dwarf Morden
3. Early Round Head
4. Early Golden Acre
5. Copenhagen Market
6. Glory of Enkhuizen
7. Danish Ballhead
8. Penn State Ballhead

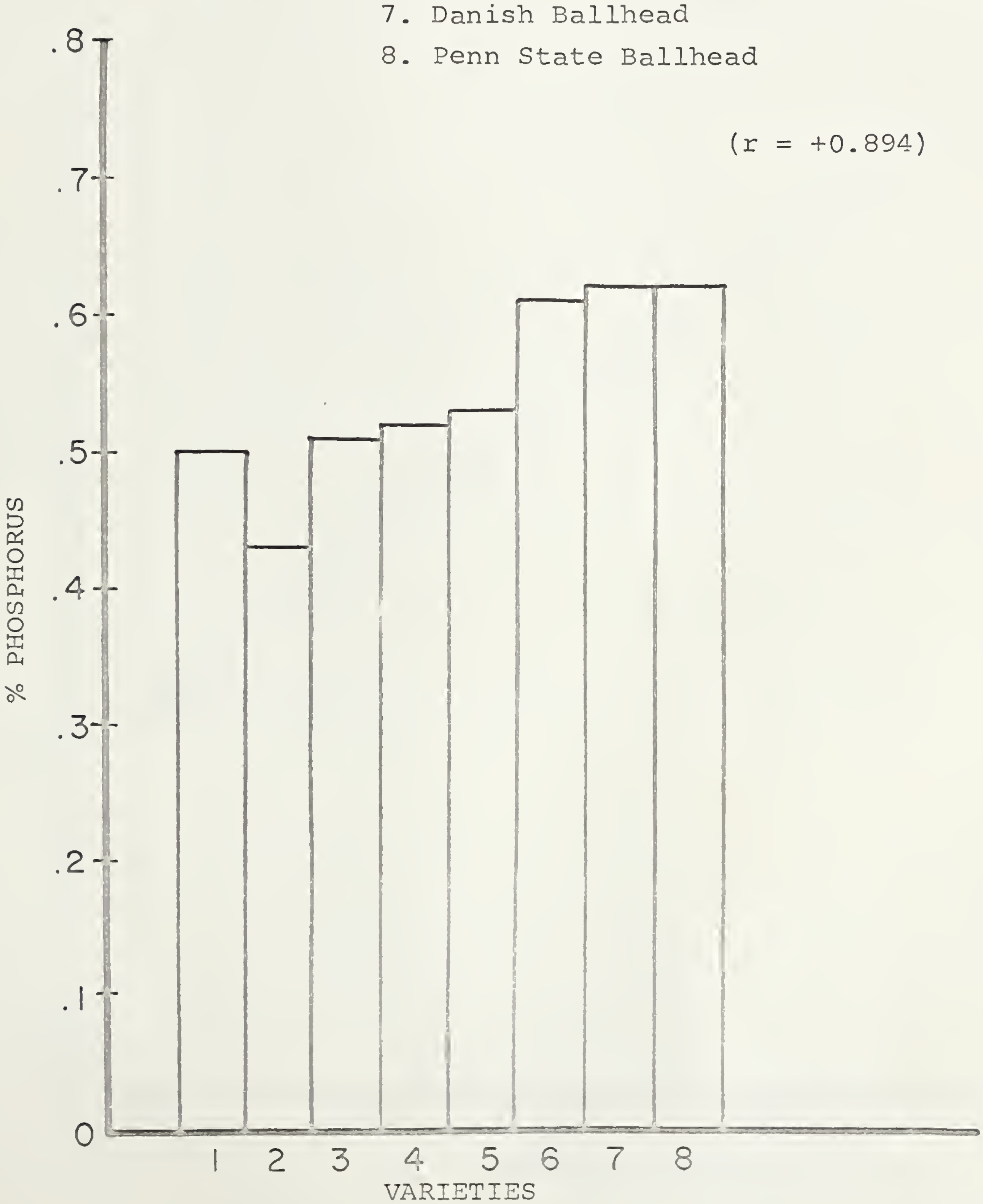


FIGURE III

Regression of phosphorus content of leaf tissue of <u>Brassica</u> <u>oleracea</u> var. <u>capitata</u> L. ten weeks after germination on days to maturity.

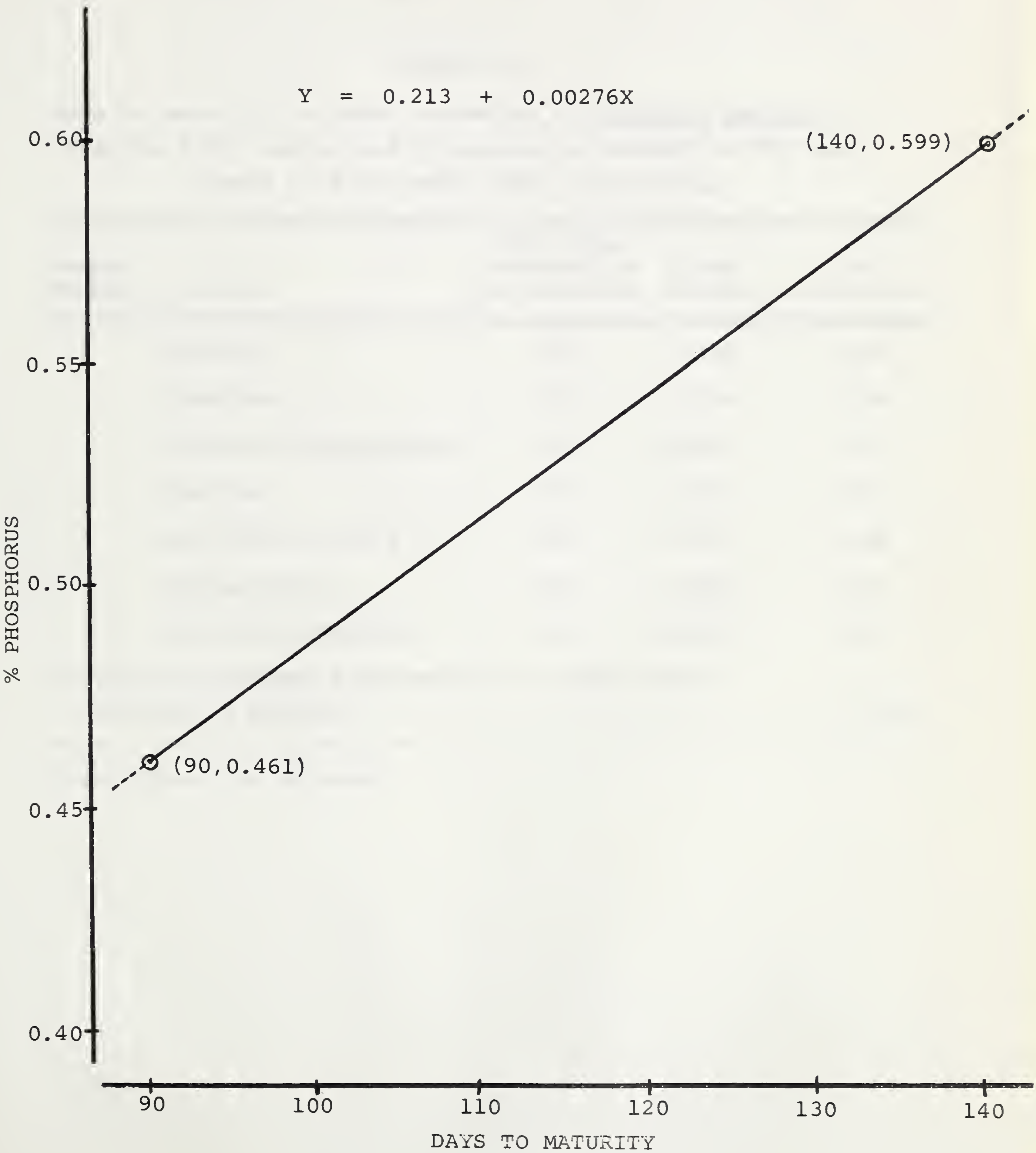


TABLE III

Days to maturity of seven varieties of Raphanus sativus L., average % dry matter and % phorphorus content in dry leaf tissue at five weeks after germination

Sample Number	Variety	Days from Germination to Maturity	% Dry Matter	% Phosphorus
1	Cavalier	35	9.348	0.66
2	Champion	37	9.344	0.48
3	Forcing Scarlet Globe	40	10.874	0.66
4	Sparkler	42	9.245	0.76
5	Long White Icicle	50	9.027	0.85
6	Chinese Rose	68	9.468	0.84
7	Long Black Spanish	74	10.265	0.90
Correlation between % phosphorus in leaf tissue and days to maturity				+0.788*

*Significant at 5% level.

FIGURE IV

Percentage Phosphorus in leaf tissue of seven varieties of field grown <u>Raphanus sativus</u> five weeks after germination.

1. Cavalier
2. Champion
3. Forcing Scarlet Globe
4. Sparkler
5. Long White Icicle
6. Chinese Rose
7. Long Black Spanish

(r = +0.788)

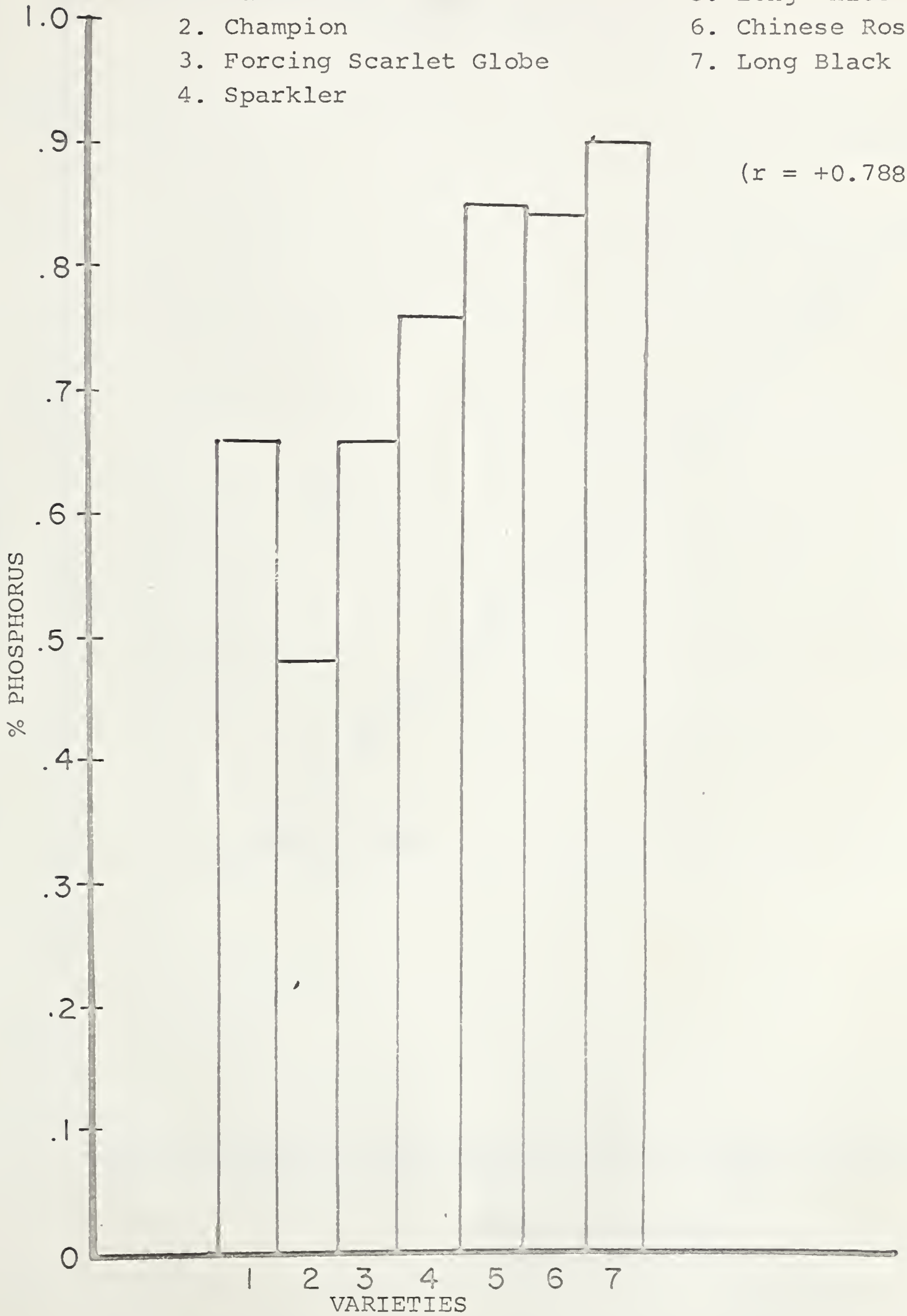
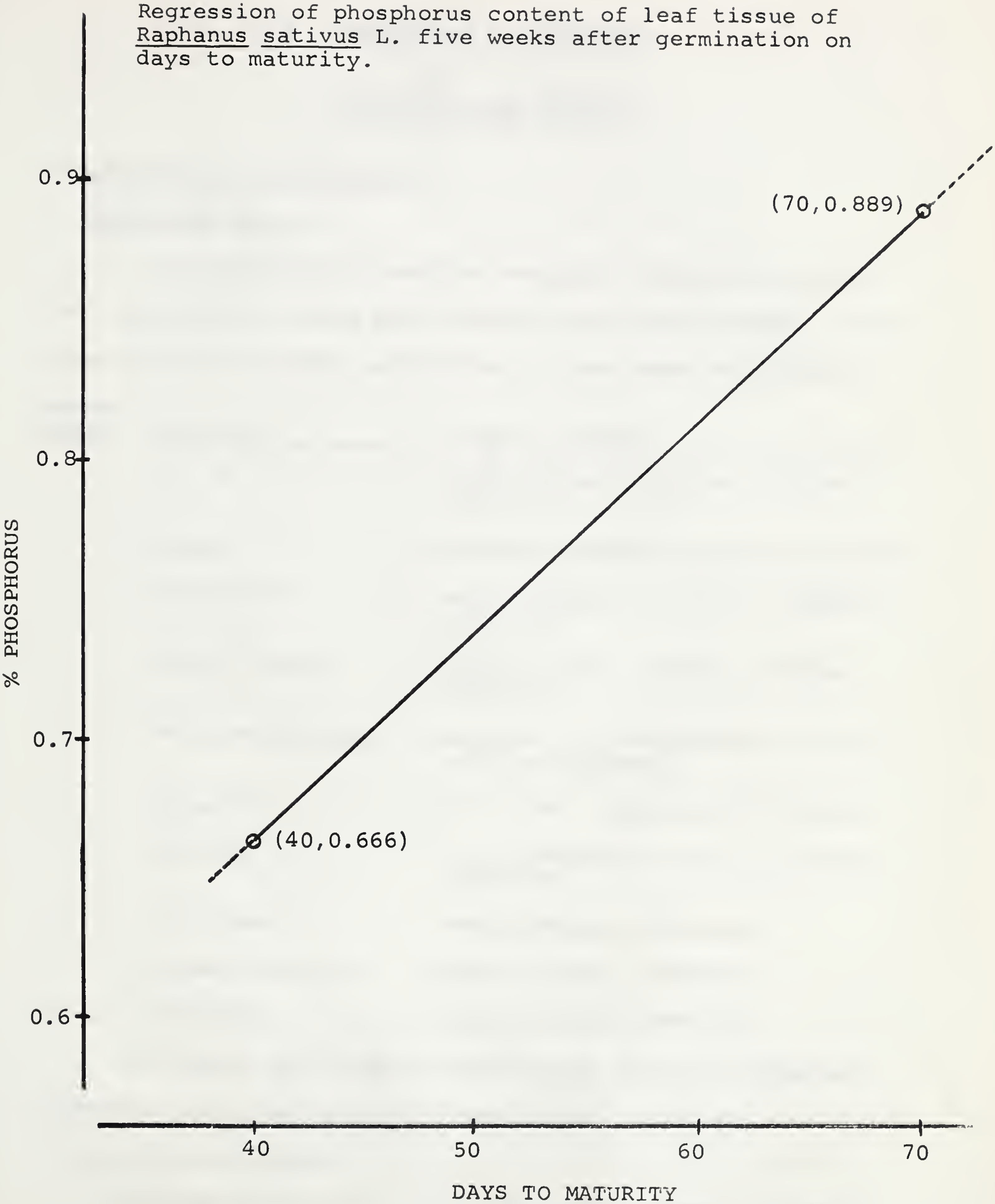


FIGURE V

Regression of phosphorus content of leaf tissue of *Raphanus sativus* L. five weeks after germination on days to maturity.



B. GREENHOUSE EXPERIMENTS

MATERIALS AND METHODS

I. *Lycopersicon esculentum* L.

Experiment No. 1

Ten varieties of tomatoes varying in maturity period from very early to late were selected for these studies. The names of the varieties and sources of seed were as follows:

Sample Number	Variety	Source of Seed
1	B.V. 5	Horticultural Station, Brooks, Alberta
2	Rocket	Research Station, Lacombe, Alberta
3	Earlinorth	A.E. McKenzie Co. Ltd., Seedsmen, Edmonton
4	Johnny Jumpup	Horticultural Station, Brooks, Alberta
5	Early Lethbridge	Division of Horticulture, University of Alberta
6	Alpha #5	Clow Seeds, Salinas, California
7	Manitoba	A.E. McKenzie Co. Ltd., Seedsmen, Edmonton
8	Bonny Best	Steele Briggs, Edmonton
9	Crimson Cushion	Steele Briggs, Edmonton
10	Beefsteak	Steele Briggs, Edmonton

The seeds were sown in vermiculite and the subsequent procedures for growing the plants were as previously described for the lettuce plants.

The depth of soil in the greenhouse benches, selected for tomato growing, was about twelve inches. Before

transplanting soil samples were taken and analysed for available nutrients. The benches had been fertilized very heavily the previous season with the result that soil tests showed very high amounts of sulphates (400 ppm) and nitrates (210 ppm) in the soil extract. Hence it was essential to leach the benches before setting the tomato plants. After leaching the soil test results were satisfactory. The nitrate level was reduced to 7 - 9 ppm, phosphate level did not change from 4 - 7 ppm and availability of potassium increased from 10 ppm to about 20 ppm. This increase in available potassium after leaching was to be expected since the pH was raised from the 5.0 - 5.3 range to 5.7 - 6.0. The conductance of soil extract was reduced from 4 - 4.5 mohs to 1.1 - 1.2 mohs as a result of leaching.

The plants were set in benches in randomized block design with two replications. The planting distance was 46 x 42 cm with two plants per treatment. Fertilizers were applied regularly according to Wiebe's (49) schedule for growing a spring tomato crop in the greenhouse.

Leaf tissue samples were taken eight weeks after germination by the method of Ward and Smith (8, 47) with slight modification. The tissue samples consisted of the sixth leaf (instead of fifth as selected by Ward) from the growing tip which is usually the first fully expanded leaf and included the whole rachis, both laminar and petiole tissue.

The drying and analytical procedures for phosphorus determinations were as previously discussed.

Experiment No. 2

The experiment with tomatoes was repeated during the autumn of 1965. Alpha #5, included in the first experiment was ommitted in the second test and two very late varieties Ponderosa and Pearson received from Dessert Seed Company, El Centro, California, were added.

The procedure for raising seedlings was as previously discussed except that they were pricked out into 3½ inches diameter earthen pots instead of veneer bands.

The plants were set in greenhouse benches in randomized block design with two replications. Fertilizers were again applied according to schedule of Wiebe (49) for autumn greenhouse tomato crop.

Starting the sixth week after germination leaf tissue samples were taken at intervals of two weeks to ascertain the changes in phosphorus level in leaf tissue at different stages of growth.

II. *Lactuca sativa* var. *capitata* L.

Experiment No. 1

The varieties were the same as in the field experiment and seedlings were raised in the same manner. They were set in greenhouse benches on May 5, 1965 in a randomized block design with two replications of four plants per treatment. The application of fertilizers after final setting was based on the recommendation of Wiebe (49).

The leaf tissue samples were taken six weeks after germination. Dry weight and phosphorus content in the leaf tissue were determined by the methods previously described.

Experiment No. 2

Another greenhouse experiment was conducted with lettuce during the autumn of 1965. The same varieties were set out in randomized block design with three replications. The method of raising seedlings and final setting was similar to those described in Experiment No. 1.

Starting the fourth week after germination leaf tissue samples were taken at intervals of two weeks to determine the fluctuations in phosphorus content in leaf tissue at various growth stages. Sampling and analytical procedures were as previously described.

III. *Brassica oleracea* var. *capitata* L.

The eight varieties used in this test were the same as described for the field experiments. Plants were set in greenhouse benches on November 4, 1965. A randomized block design with three replications of three plants per treatment was used. The planting distance was 52 x 40 cm . Before final setting soil samples were taken and analysed for available nutrients.

The leaf tissue samples were taken at intervals of two weeks starting the fifth week after germination.

IV. Raphanus sativus L.

The varieties were the same as those grown at the Parkland Field Laboratory with the addition of the variety White Strasburg received from Dessert Seed Co., El Centro, California.

The seeds were sown in the greenhouse during the autumn of 1965 in randomized block design with two replications. After germination fertilizers were applied at the rate recommended by Wiebe (49).

The leaf tissue samples were taken at 15 days, 25 days and 35 days after germination to determine the changes in phosphorus content leaf tissue at various stages of growth. The leaf tissue samples were dried and analysed for phosphorus content.

RESULTS

I. Tomatoes

There were no differences in dry matter content between early and late varieties of the spring experiment when the plants were sampled eight weeks after germination. The phosphorus content in earlier varieties was much higher than in later ones. As summarized in Table IV and Figure VI, the early variety B.V. 5 had about 55% more phosphorus in the leaf tissue than did the late variety Beefsteak. The correlation of -0.765 between percentage phosphorus in leaf tissue and days to maturity was significant at the 1% level. Figure VII illustrates regression of phosphorus content in leaf tissue on days to maturity and the regression line has a slope of 0.00862.

In the second experiment also there were no differences in dry matter among various varieties at different stages of growth as indicated in Table V. The earlier varieties had more phosphorus in leaf tissue than did the later ones at six and eight weeks after germination. The differences in phosphorus level among various varieties were significant at the 1% level at all four stages of growth as summarized in Table VI. As evident in Figures VIII and IX, the correlation between percentage phosphorus in leaf tissue and days to maturity was significant at the 1% level six and eight weeks after germination, whereas there was no significant correlation ten and twelve weeks after germination (Figures X and XI). Duncan's new multiple range test (45) also indicated significant differences in phosphorus content between varieties of various maturity groups at the first two sampling stages. Figure XII shows regression of phosphorus on days to maturity six and eight weeks after germination with regression coefficient of -0.00752 and -0.0046 respectively.

TABLE IV

Days to maturity of ten varieties of *Lycopersicon esculentum* L., average % dry matter and % phosphorus content in dry leaf tissue at eight weeks after germination

Sample Number	Variety	Days from Germination to Maturity of First 3 Fruits	% Dry Matter	% Phosphorus
1	B.V. 5	96	9.97	1.18
2	Rocket	97	9.51	1.11
3	Earlinorth	100	10.03	0.95
4	Johnny Jumpup	100	9.36	1.00
5	Early Lethbridge	107	9.25	0.93
6	Alpha #5	113	10.44	0.74
7	Manitoba	116	9.11	0.78
8	Bonny Best	118	9.32	1.04
9	Crimson Cushion	130	9.50	0.82
10	Beefsteak	138	10.44	0.70
Correlation between % phosphorus in leaf tissue and days to maturity				-0.765**

**Significant at 1% level.

FIGURE VI

Percentage Phosphorus in leaf tissue of ten varieties of greenhouse grown _Lycopersicon esculentum_ eight weeks after germination.

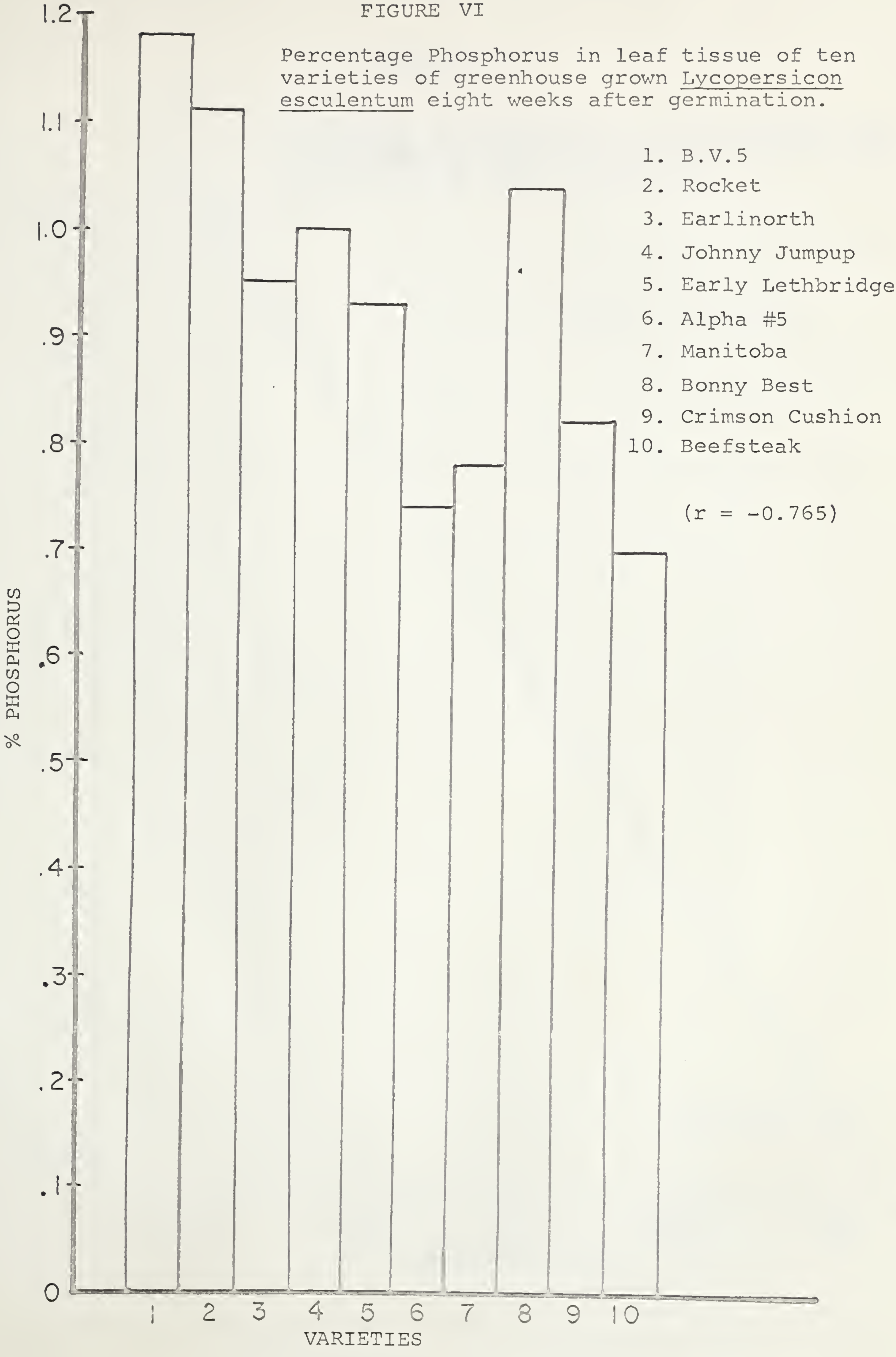
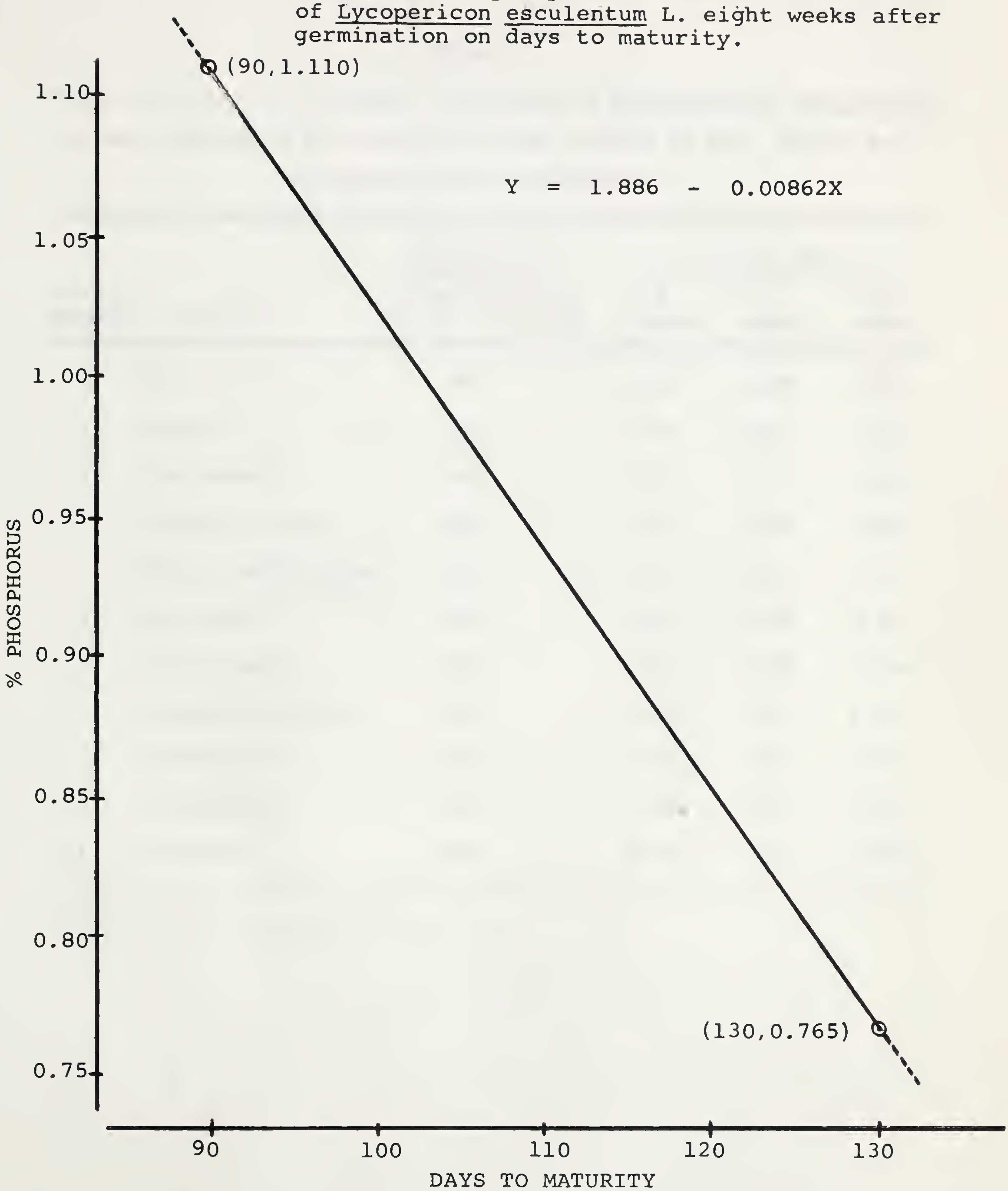


FIGURE VII

Regression of phosphorus content of leaf tissue of Lycopericon esculentum L. eight weeks after germination on days to maturity.



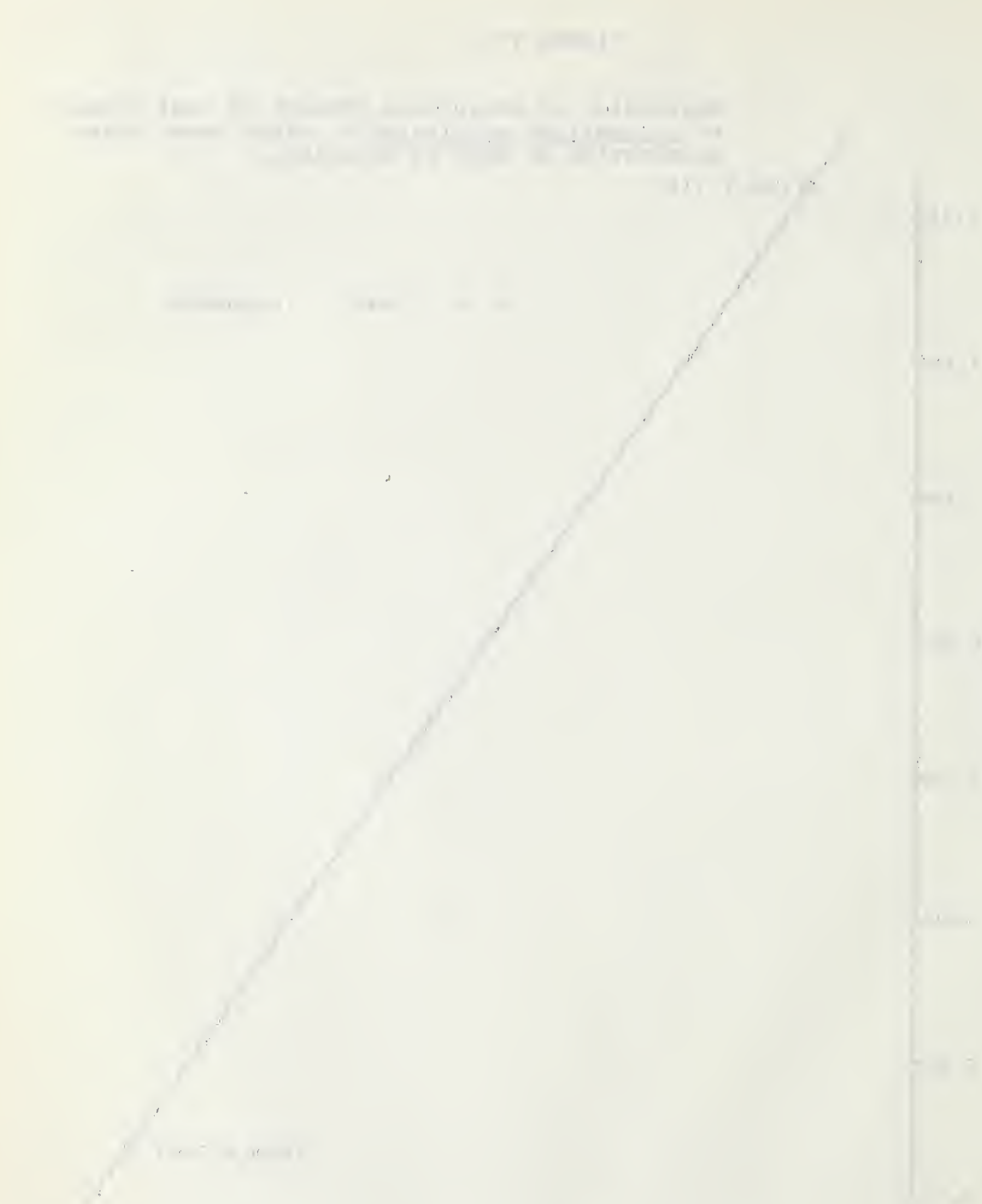


TABLE V

Days to maturity of eleven varieties of *Lycopersicon esculentum* L. and average % dry matter in leaf tissue at six, eight and ten weeks after germination

Sample Number	Variety	Days from Germination to Maturity of First 3 Fruits	% Dry Matter 6 Weeks	% Dry Matter 8 Weeks	% Dry Matter 10 Weeks
1	B.V. 5	96	9.68	7.08	6.24
2	Rocket	97	8.35	6.66	7.04
3	Earlinorth	100	8.56	7.30	6.66
4	Johnny Jumpup	100	7.93	6.46	5.95
5	Early Lethbridge	107	7.78	6.57	6.33
6	Manitoba	116	7.28	5.95	5.85
7	Bonny Best	118	7.57	5.98	5.78
8	Crimson Cushion	130	8.53	7.06	6.64
9	Beefsteak	138	8.76	7.01	6.98
10	Ponderosa	144	8.96	7.59	6.48
11	Pearson	147	8.36	7.13	6.95

Differences not significant.

TABLE VI

Days to maturity of eleven varieties of *Lycopersicon esculentum* L. and average % phosphorus content in dry leaf tissue at six, eight, ten and twelve weeks after germination

Sample Number	Variety	Days from Germination to Maturity of First 3 Fruits	% Phosphorus 6 Weeks	% Phosphorus 8 Weeks	% Phosphorus 10 Weeks	% Phosphorus 12 Weeks
1	B.V. 5	96	1.86^{a} [1]	1.55^{ab} [1]	1.30^{de} [1]	1.23^{ef} [1]
2	Rocket	97	1.86^{a}	1.57^{a}	1.35^{cd}	1.28^{cdef}
3	Earlinorth	100	1.70^{abc}	1.54^{b}	1.41^{bc}	1.39^{bcde}
4	Johnny Jumpup	100	1.73^{ab}	1.46^{c}	1.28^{de}	1.48^{abcd}
5	Early Lethbridge	107	1.63^{bcd}	1.40^{d}	1.62^{ab}	1.54^{ab}
6	Manitoba	116	1.55^{cd}	1.41^{d}	1.70^{a}	1.73^{a}
7	Bonny Best	118	1.55^{cd}	1.35^{e}	1.32^{de}	1.26^{def}
8	Crimson Cushion	130	1.48^{de}	1.38^{d}	1.66^{a}	1.34^{bcdef}
9	Beefsteak	138	1.58^{bcd}	1.36^{e}	1.54^{abc}	1.76^{a}
10	Ponderosa	144	1.73^{ab}	1.54^{b}	1.68^{a}	1.53^{abc}
11	Pearson	147	1.39^{e}	1.29^{f}	1.18^{e}	1.16^{f}
Correlation between % phosphorus in leaf tissue and days to maturity			-0.891**	-0.88**	N.S.	N.S.

F values for % phosphorus at six, eight, ten and twelve weeks was significant at 1% level.

**Significant at 1% level.

[1]Numbers in each column which are not followed by the same letter are significantly different from each other at 5% level of significance as judged by Duncan's new multiple range test.

FIGURE VIII

Percentage Phosphorus in leaf tissue of ten varieties of greenhouse grown <u>Lycopersicon esculentum</u> six weeks after germination.

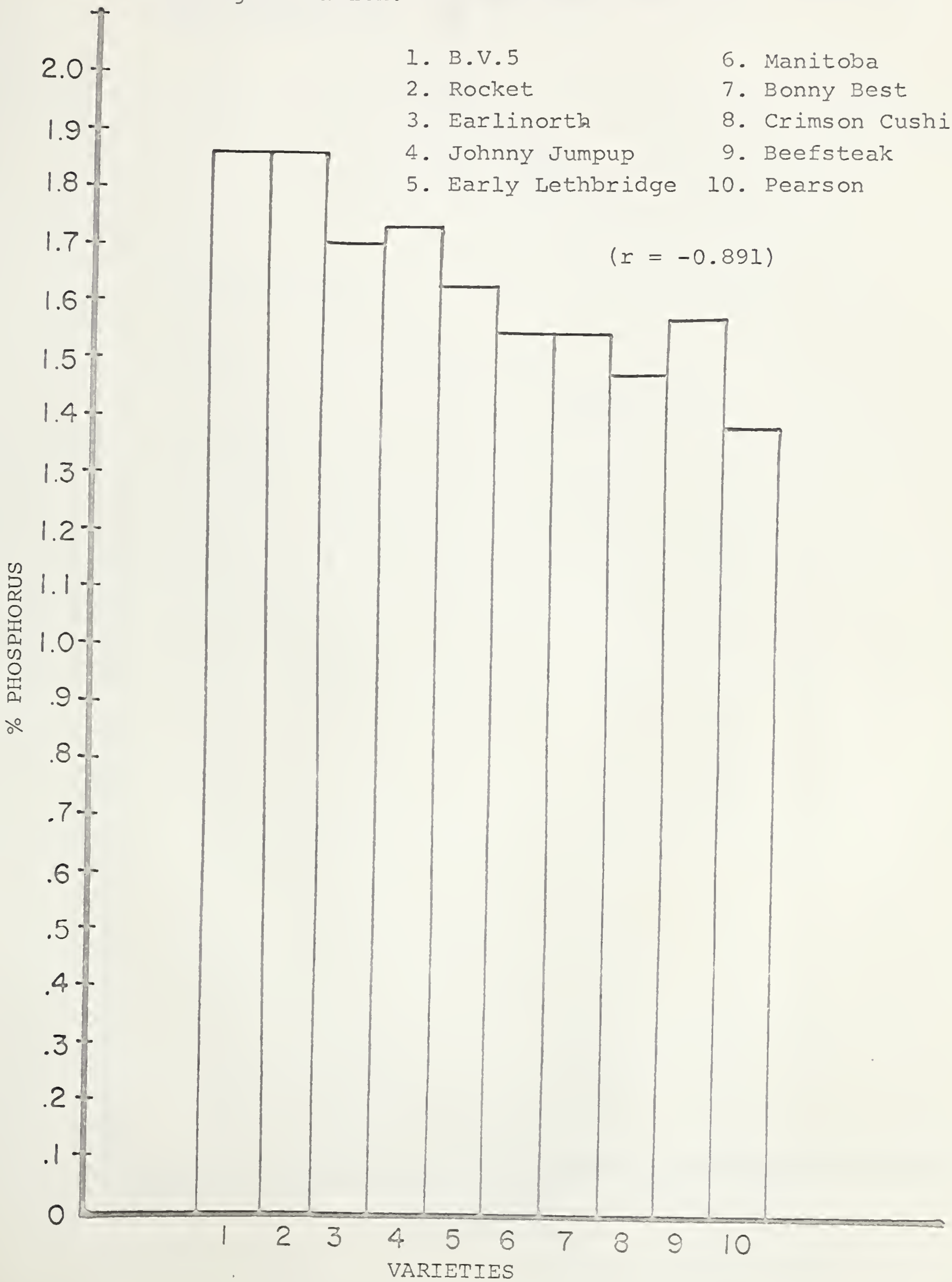


FIGURE IX

Percentage Phosphorus in leaf tissue of ten varieties of greenhouse grown Lycopersicon esculentum eight weeks after germination.

1. B.V.5
2. Rocket
3. Earlinorth
4. Johnny Jumpup
5. Early Lethbridge
6. Manitoba
7. Bonny Best
8. Crimson Cushion
9. Beefsteak
10. Pearson

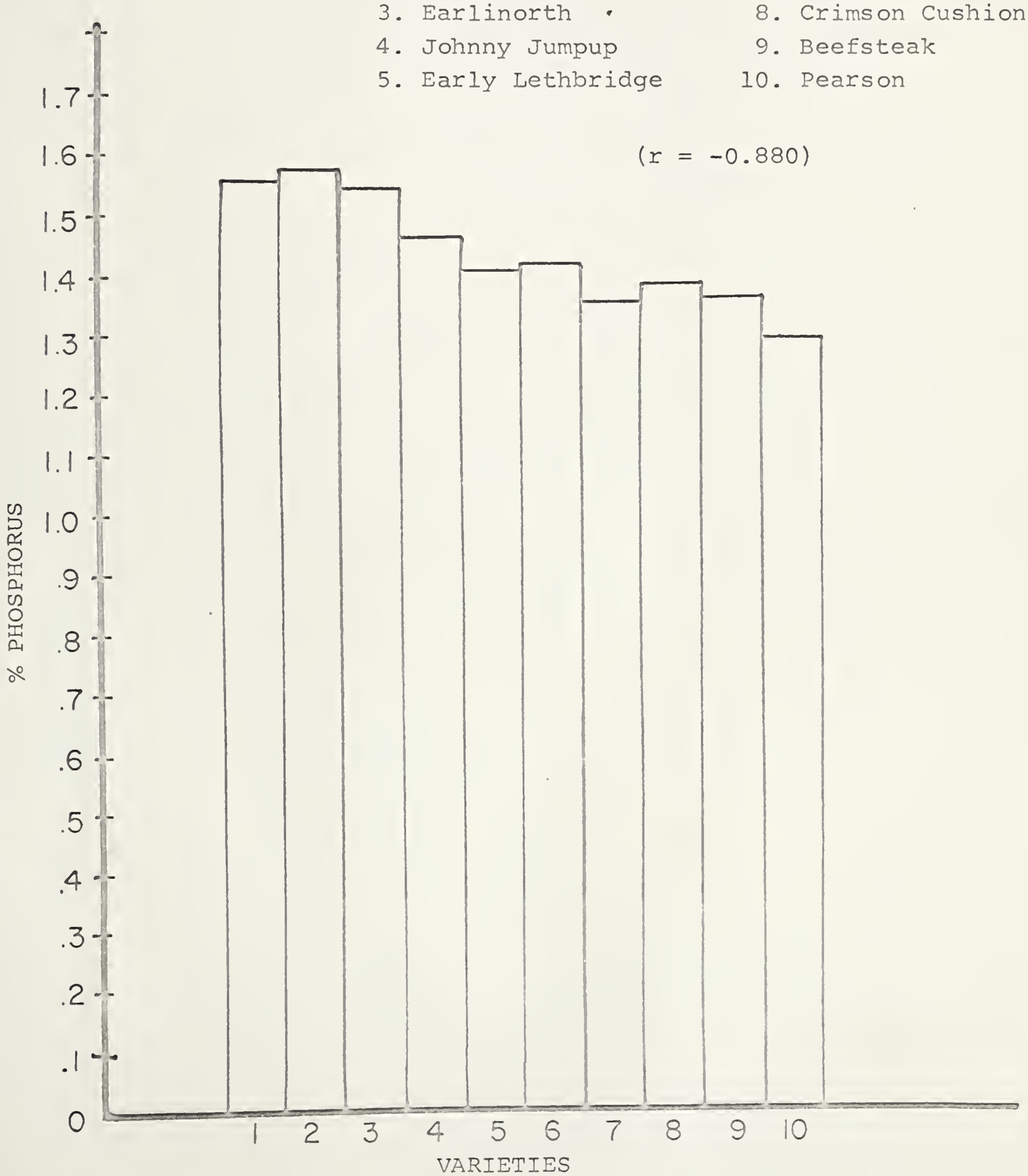


FIGURE X

Percentage Phosphorus in leaf tissue of ten varieties of greenhouse grown <u>Lycopersicon</u> <u>esculentum</u> ten weeks after germination.

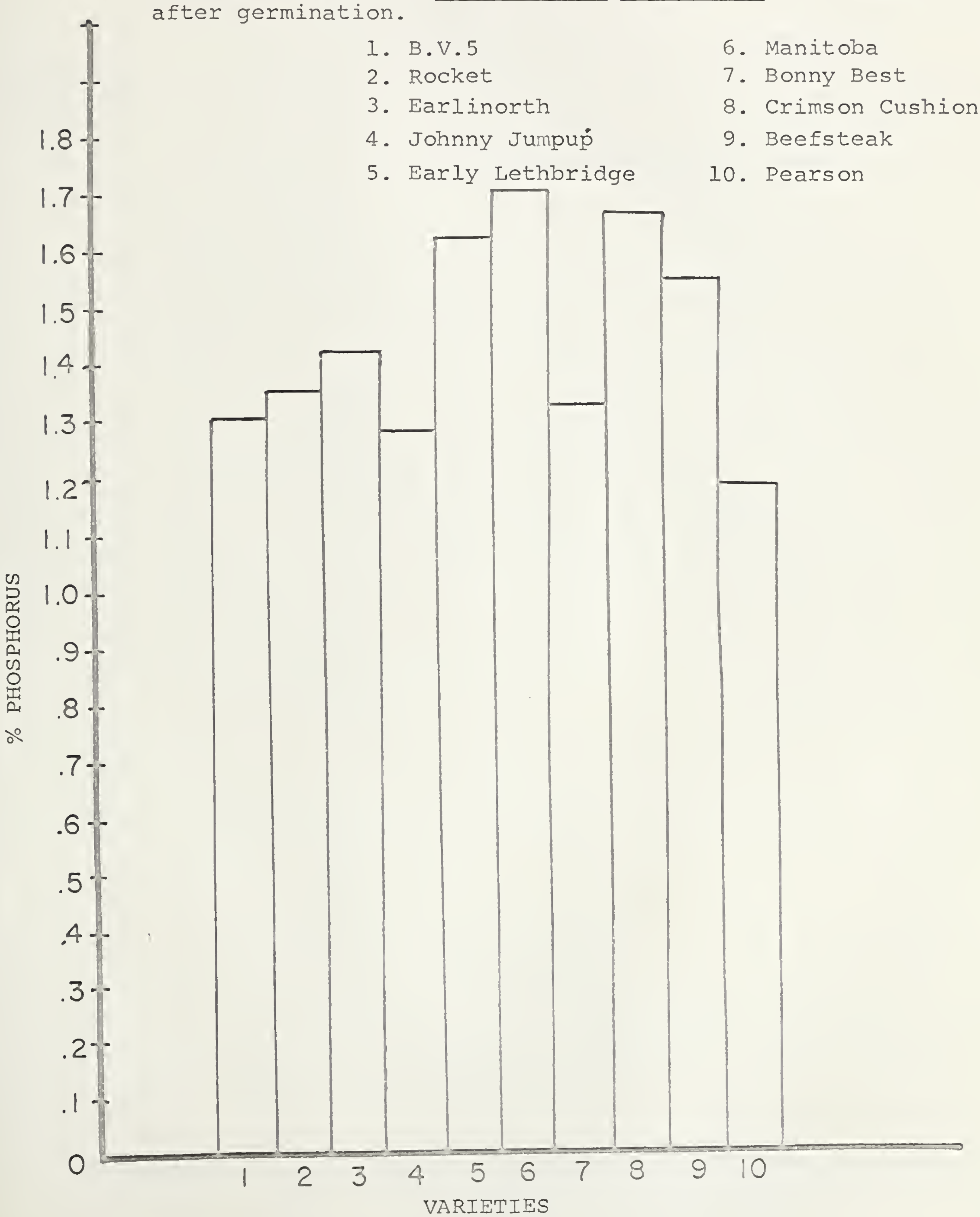


FIGURE XI

Percentage Phosphorus in leaf tissue of ten varieties of greenhouse grown Lycopersicon esculentum 12 weeks after germination.

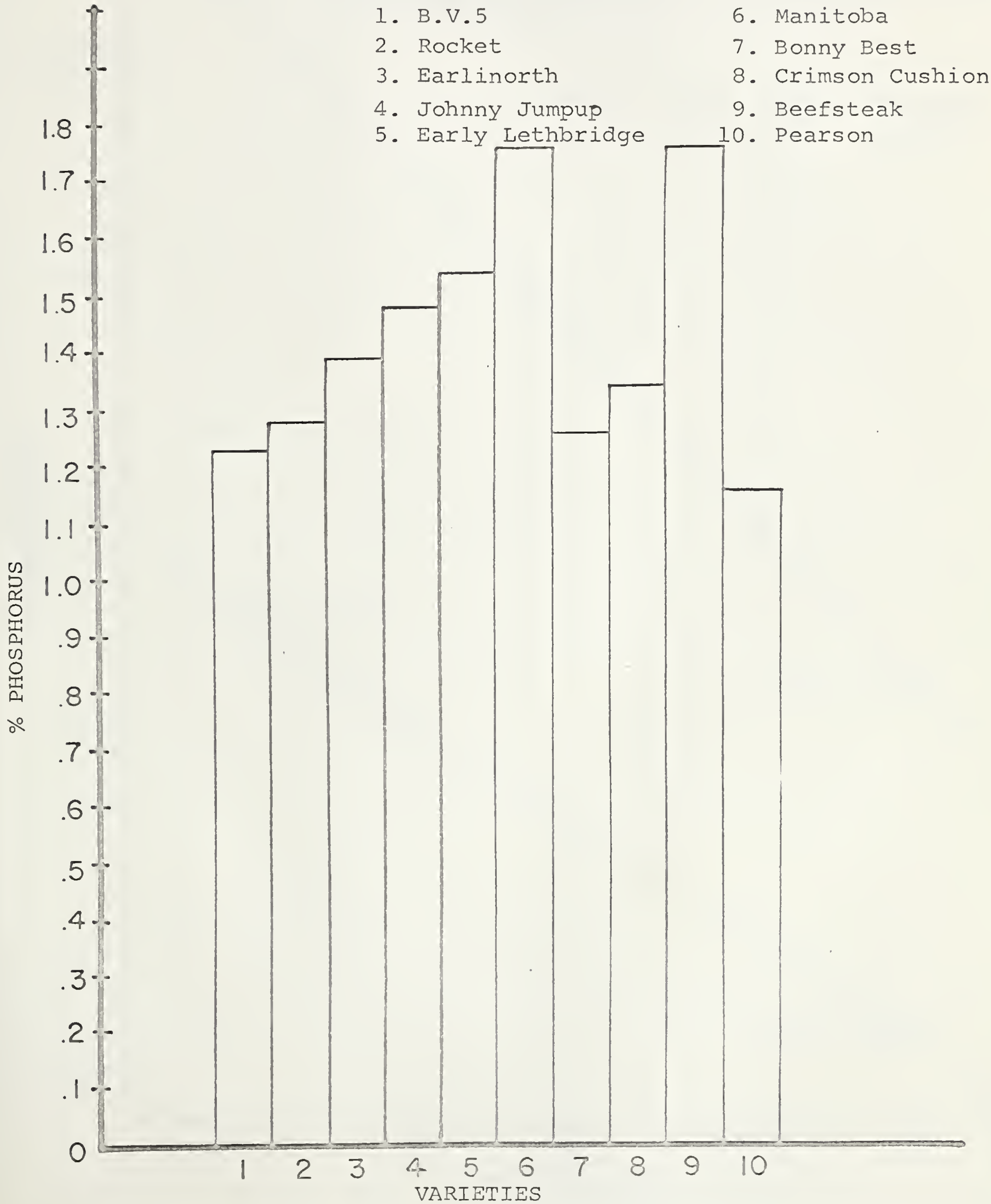
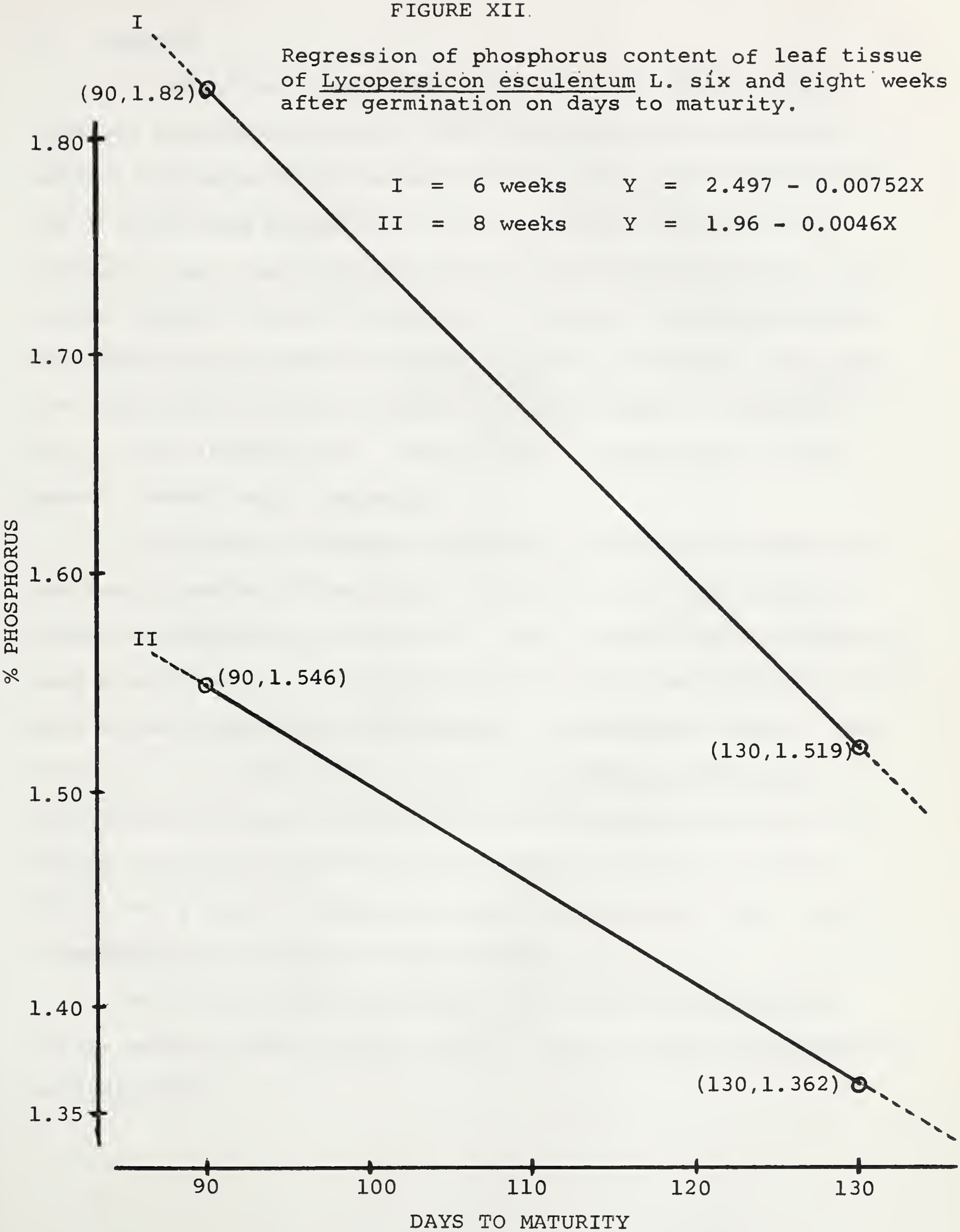


FIGURE XII

Regression of phosphorus content of leaf tissue of Lycopersicon esculentum L. six and eight weeks after germination on days to maturity.



II. Lettuce

In the first greenhouse experiment the earlier varieties had more phosphorus in leaf tissue than later ones six weeks after germination as seen in Table VII and Figure XIII. The F tests were significant at the 1% level. Duncan's new multiple range test also showed significant differences in phosphorus content between varieties of different maturity periods. The correlation between phosphorus content and days to maturity was significant at the 5% level with the slope of regression being 0.016 (Figure XIV). There were no differences in dry matter content among varieties.

In a second greenhouse experiment earlier varieties also had more phosphorus than later ones at all the four stages of growth as summarized in Table IX. The F values for % phosphorus were significant at the 1% level and Duncan's new multiple range test showed significant differences in phosphorus content among varieties. The correlation between percentage phosphorus in leaf tissue and days to maturity were significant at all four stages (Figures XV and XVI) with regression shown in Figure XVII. The slope of regression was steepest at the eight week stage when correlation was also highest.

As in the first experiment there were no differences in dry matter content at any stage. These results are presented in Table VIII.

TABLE VII

Days to maturity of six varieties of _Lactuca_ _sativa_ var. _capitata_ L., average % dry matter and % phosphorus in dry leaf tissue at six weeks after germination

Sample Number	Variety	Days from Germination to 60% Marketable Heads	% Dry Matter	% Phosphorus
1	New York 12	77	5.48	0.97 a [1]
2	Pennlake	82	5.23	0.82 b
3	Great Lakes 659	83	5.63	0.76 c
4	Premier Great Lakes	86	6.02	0.71 cd
5	Francisco	92	5.60	0.64 e
6	Alaska	95	5.33	0.68 de
Correlation between % phosphorus in leaf tissue and days to maturity				-0.906*

F values for % phosphorus in leaf tissue was significant at 1% level.

*Significant at 5% level.

[1] Numbers in the column which are not followed by the same letter are significantly different from each other at 5% level of significance as judged by Duncan's new multiple range test.

FIGURE XIII

Percentage Phosphorus in leaf tissue of six varieties of greenhouse grown <u>Lactuca</u> <u>sativa</u> var. <u>capitata</u> six weeks after germination.

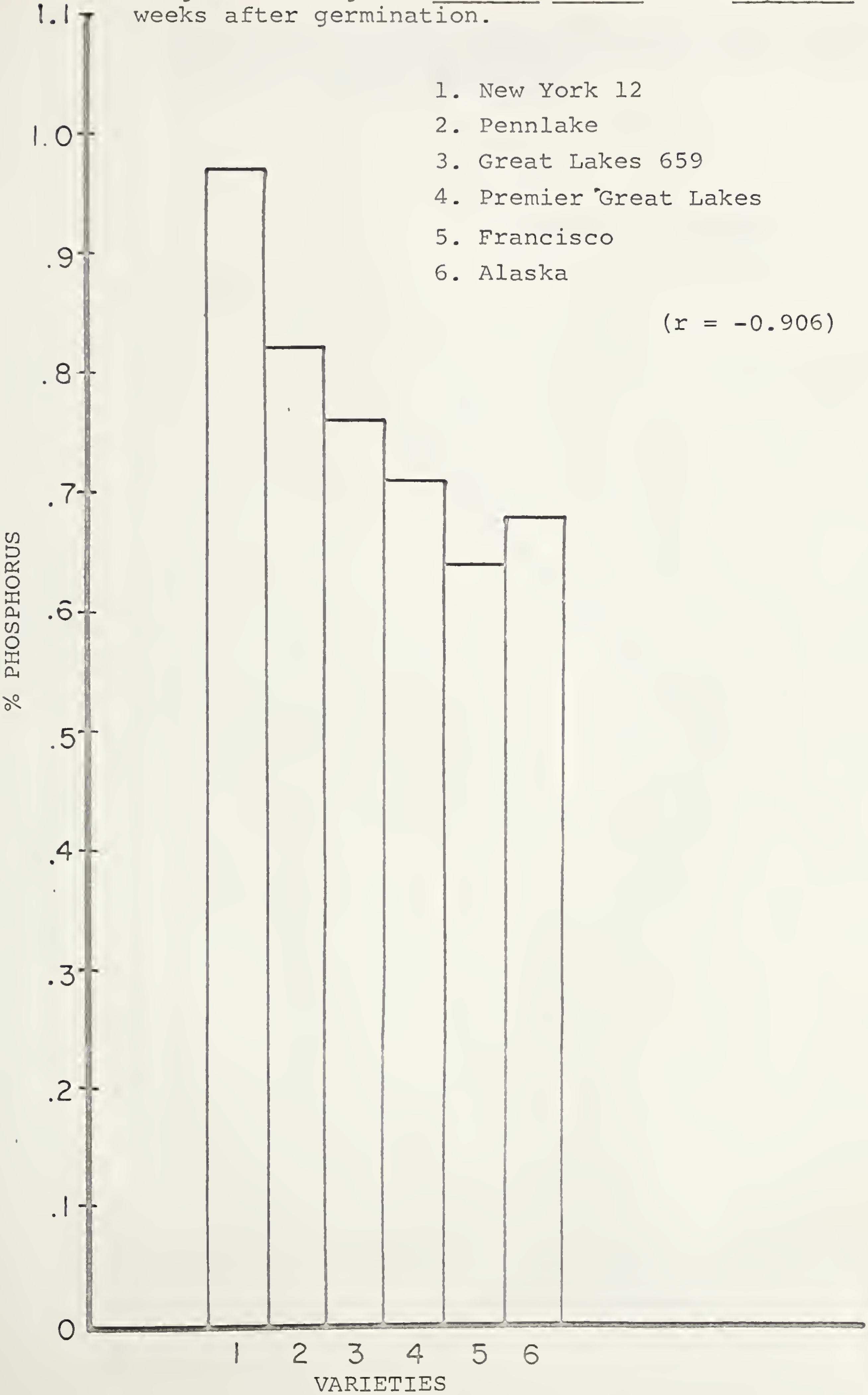


FIGURE XIV

Regression of phosphorus content of leaf tissue of *Lactuca sativa* var. *capitata* L. six weeks after germination on days to maturity.

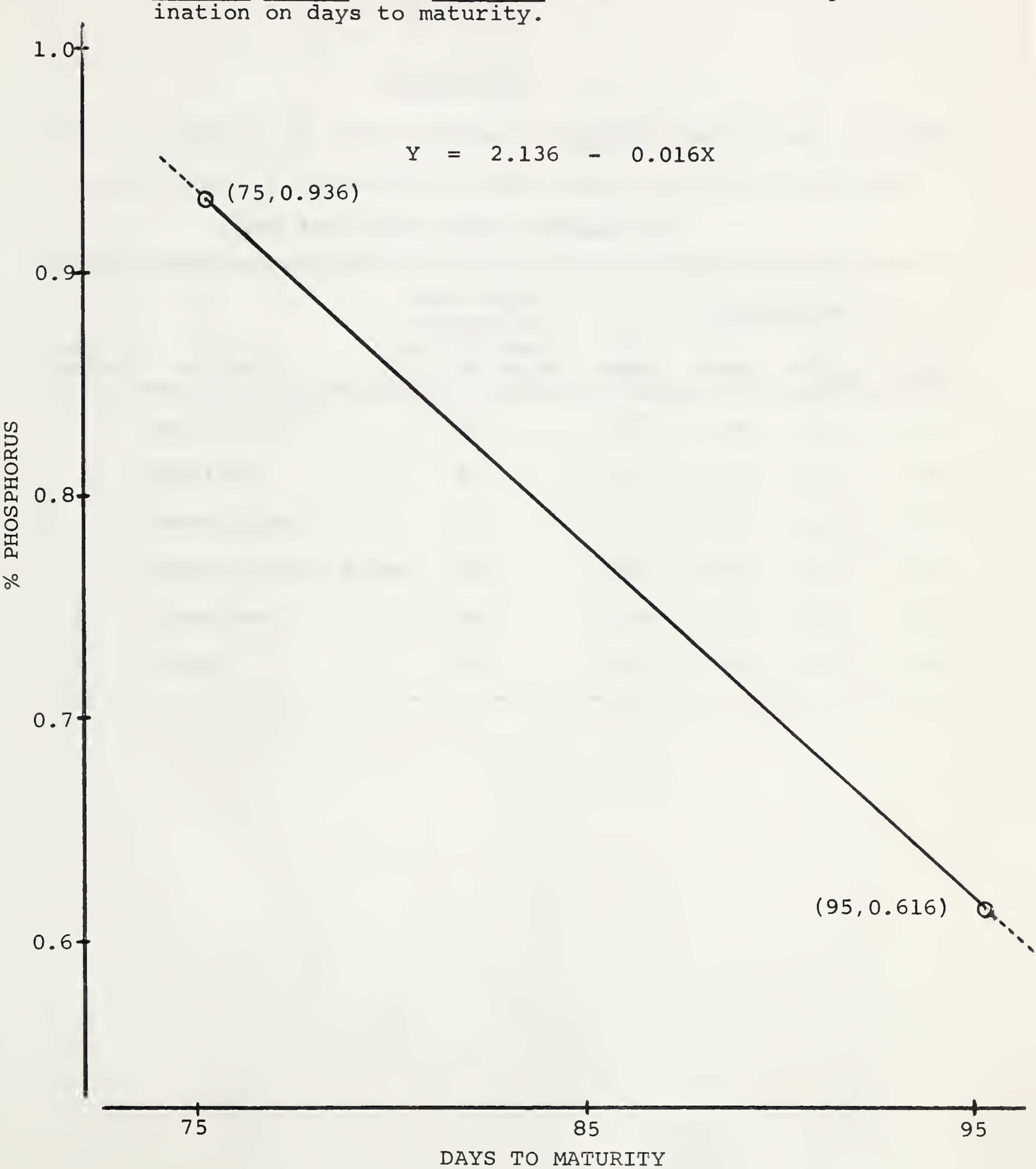


TABLE VIII

Days to maturity of six varieties of Lactuca sativa var. capitata L. and average % dry matter in leaf tissue at four, six, eight and ten weeks after germination

Sample Number	Variety	Days from Germination to 60% Marketable Heads	% Dry Matter 4 Weeks	6 Weeks	8 Weeks	10 Weeks
1	New York 12	77	4.67	5.85	5.54	4.62
2	Pennlake	82	4.37	4.78	4.97	4.68
3	Great Lakes 659	83	4.46	5.41	5.65	4.74
4	Premier Great Lakes	86	4.87	5.41	5.05	4.62
5	Francisco	92	4.44	5.57	5.14	4.39
6	Alaska	95	4.37	4.86	4.81	4.45

TABLE IX

Days to maturity of six varieties of _Lactuca_ _sativa_ var. _capitata_ L. and average % phosphorus content in dry leaf tissue at four, six, eight and ten weeks after germination

Sample Number	Variety	Days from Germination to 60% Marketable Heads	% Phosphorus 4 Weeks	6 Weeks	8 Weeks	10 Weeks
1	New York 12	77	1.20^{a} [1]	1.05^{a} [1]	0.80^{a} [1]	0.75^{a} [1]
2	Pennlake	82	1.13^{b}	0.99^{ab}	0.68^{ab}	0.63^{b}
3	Great Lakes 659	83	0.95^{c}	0.82^{bc}	0.66^{abc}	0.58^{bcd}
4	Premier Great Lakes	86	0.85^{c}	0.78^{bc}	0.65^{bc}	0.61^{bc}
5	Francisco	92	0.90^{c}	0.67^{c}	0.54^{c}	0.54^{d}
6	Alaska	95	0.85^{c}	0.72^{bc}	0.58^{bc}	0.55^{d}
Correlation between % phosphorus in leaf tissue and days to maturity			-0.822*	-0.915*	-0.936**	-0.856*

F values for % phosphorus were significant at 1% level at four and ten week stage and at 5% level at six and eight weeks after germination.

*Significant at 5% level.

**Significant at 1% level.

[1] Numbers in each column which are not followed by the same letter are significantly different from each other at 5% level of significance as judged by Duncan's new multiple range test.

FIGURE XV

Percentage Phosphorus in leaf tissue of six varieties of greenhouse grown <u>Lactuca sativa</u> var. <u>capitata</u> four and six weeks after germination.

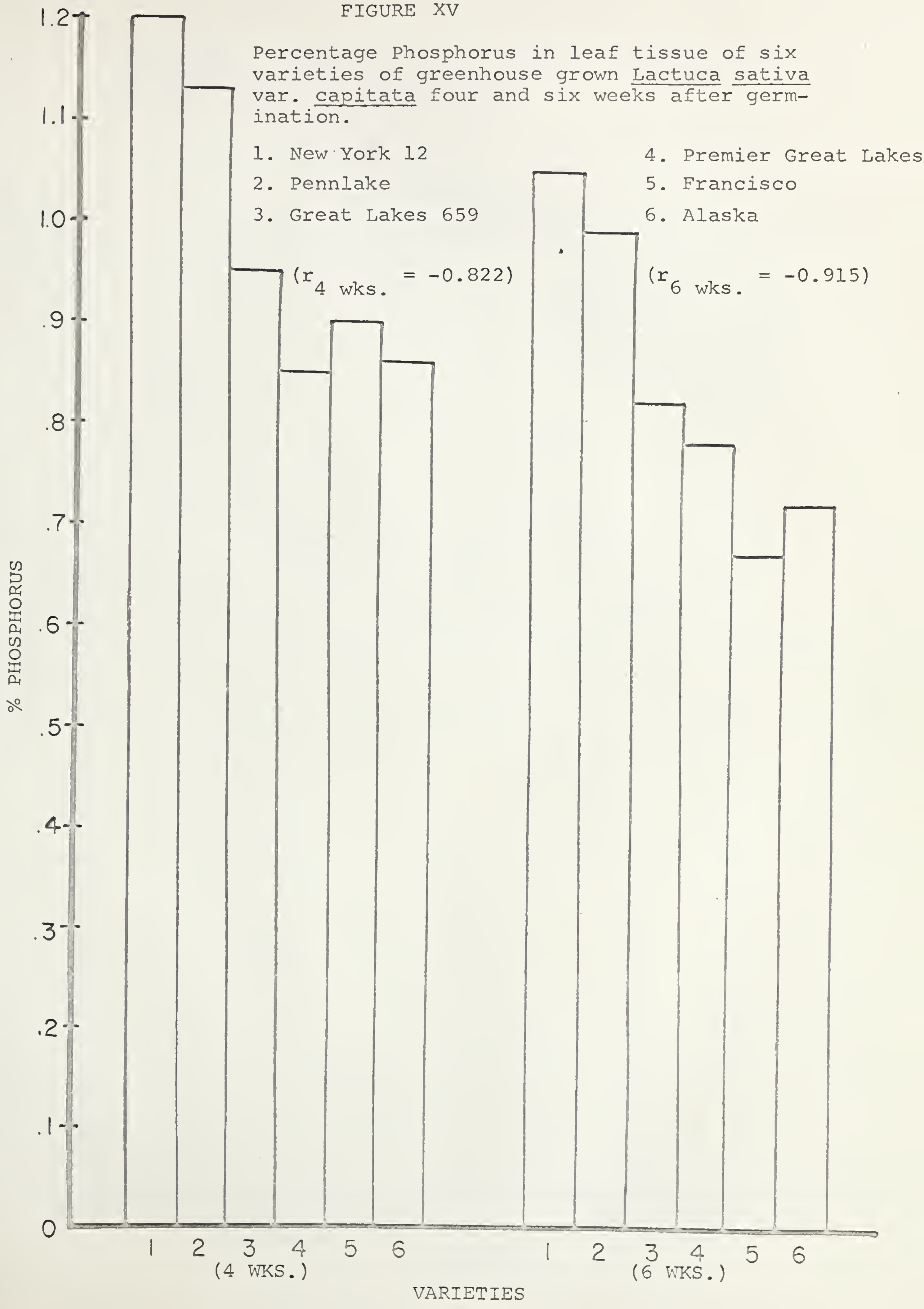


FIGURE XVI

Percentage Phosphorus in leaf tissue of six varieties of greenhouse grown Lactuca sativa var. capitata eight and ten weeks after germination.

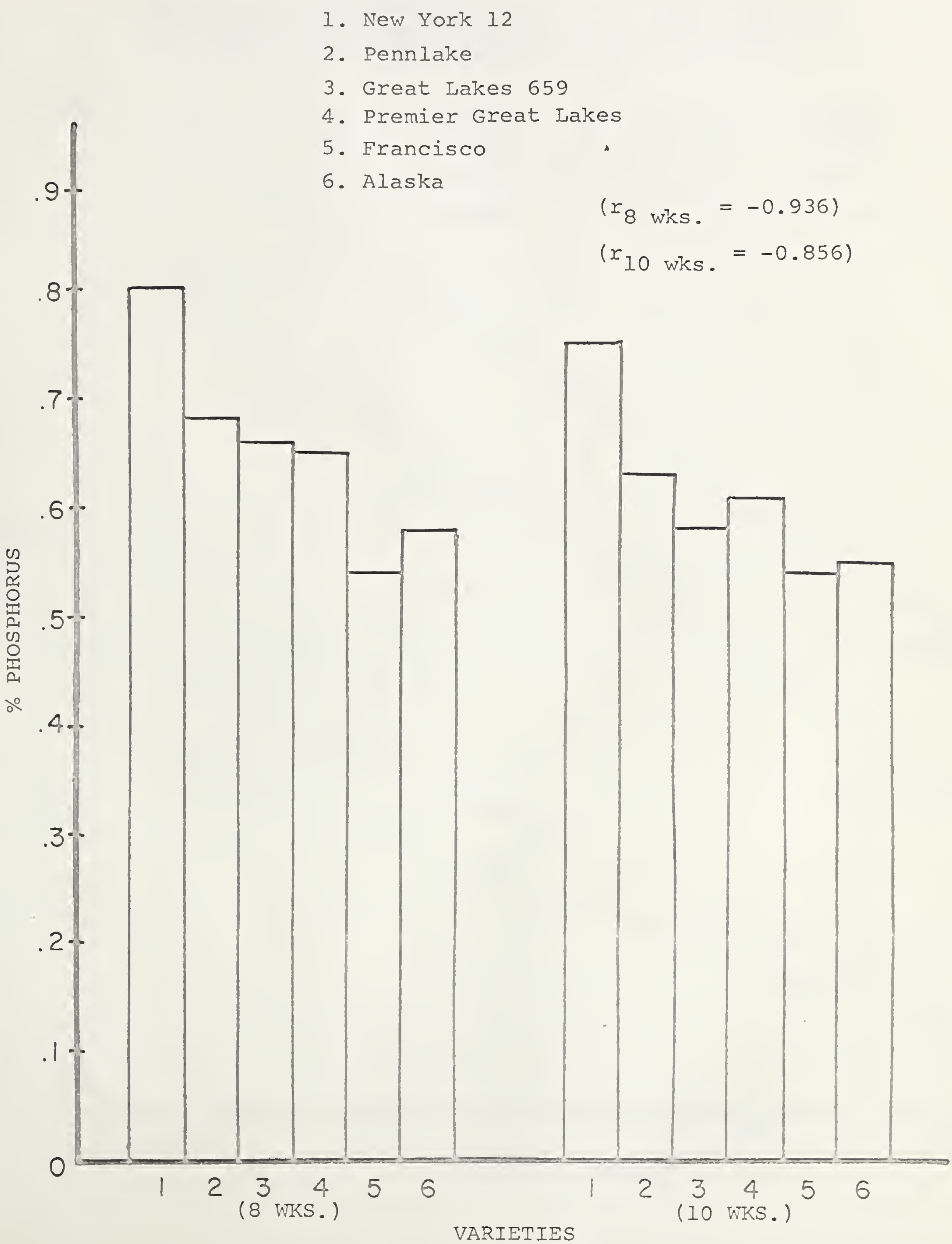
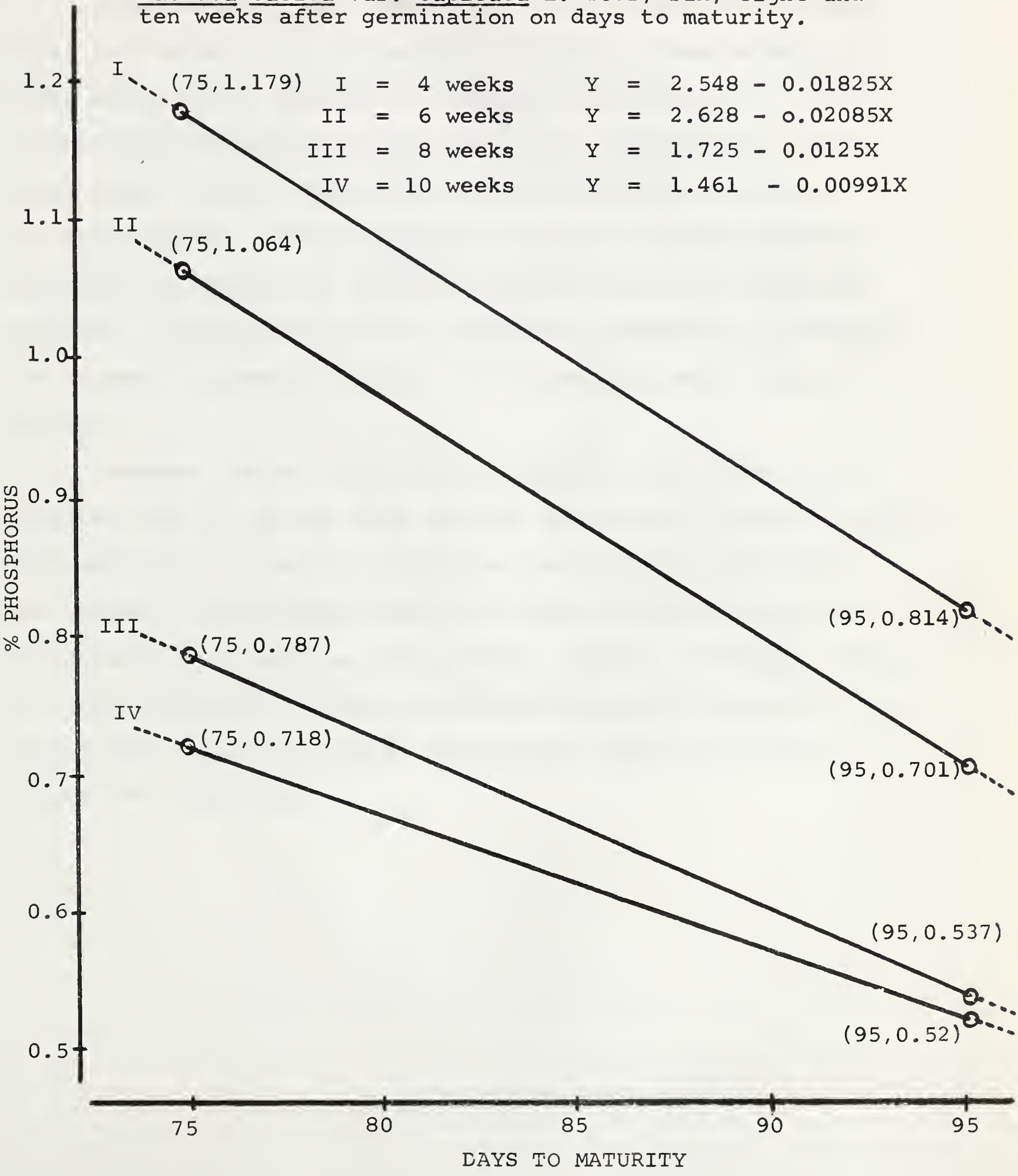


FIGURE XVII

Regression of phosphorus content of leaf tissue of *Lactuca sativa* var. *capitata* L. four, six, eight and ten weeks after germination on days to maturity.



III. Cabbage

Unfortunately the temperature in the greenhouse at bench level fell below 50° F, in spite of automatic temperature controls being set to maintain the temperature above 60° F. The light conditions were also poor even with supplementary artificial light. Light intensity on many winter days was only 250 - 400 foot candles. These conditions resulted in stem elongation and poor head formation, hence the results were not considered reliable. Further studies are, therefore, necessary to evaluate the changes in phosphorus level at different growth stages of cabbage.

However, as is illustrated in Table X, the later varieties had more dry matter than earlier ones at all stages of growth. Although the F values for phosphorus content were significant and Duncan's new multiple range test also indicated significant differences in phosphorus level between various varieties (Table XI), the correlation between phosphorus and days to maturity was significant at 13 weeks after germination only as is evident in Figures XVIII and XIX.

TABLE X

Days to maturity of eight varieties of _Brassica_ _oleracea_ var. _capitata_ L. and average % dry matter in leaf tissue at five, seven, nine and eleven weeks after germination

Sample Number	Variety	Days From Germination to 70% Marketable Heads	% Dry Matter			
			5 Weeks	7 Weeks	9 Weeks	11 Weeks
1	First Acre	96	6.45	6.20	5.83	5.50
2	Dwarf Morden	98	6.19	6.67	6.09	5.90
3	Early Round Head	98	6.11	6.65	5.90	5.61
4	Early Golden Acre	111	6.08	7.00	6.67	6.00
5	Copenhagen Market	115	6.46	6.77	6.48	6.03
6	Glory of Enkhuizen	135	5.97	6.61	6.32	5.94
7	Danish Ballhead	148	6.78	7.14	6.82	6.66
8	Penn State Ballhead	152	6.87	7.12	6.53	6.29

TABLE XI

Days to maturity of eight varieties of *Brassica oleracea* var. *capitata* L. and % phosphorus in dry leaf tissue at five, seven, nine, eleven and thirteen weeks after germination

Sample Number	Variety	Days from Germination to 70% Marketable Heads	% Phosphorus 5 Weeks	7 Weeks	9 Weeks	11 Weeks	13 Weeks
1	First Acre	96	0.85	0.65^{a}[1]	0.41^{a}[1]	0.46^{ab}[1]	0.59
2	Dwarf Morden	98	0.87	0.62^{ab}	0.42^{a}	0.45^{ab}	0.42
3	Early Round Head	98	0.65	0.65^{a}	0.36^{ab}	0.53^{a}	0.50
4	Early Golden Acre	111	0.65	0.50^{bc}	0.32^{ab}	0.44^{ab}	0.43
5	Copenhagen Market	115	0.49	0.45^{c}	0.32^{ab}	0.42^{b}	0.41
6	Glory of Enkhuizen	135	0.55	0.51^{bc}	0.30^{b}	0.33^{c}	0.28
7	Danish Ballhead	148	0.71	0.55^{abc}	0.34^{ab}	0.40^{bc}	0.37
8	Penn State Ballhead	152	0.73	0.54^{abc}	0.38^{ab}	0.43^{ab}	0.36
Correlation between % phosphorus in leaf tissue and days to maturity			N.S.	N.S.	N.S.	N.S.	-0.764*

F values for % phosphorus were significant at 5% level at seven and eleven weeks after germination.

*Significant at 5% level.

[1]Numbers in each column which are not followed by the same letters are significantly different from each other at 5% level of significance as judged by Duncan's new multiple range test.

FIGURE XVIII

Percentage Phosphorus in leaf tissue of eight varieties of greenhouse grown _Brassica oleracea_ var. _capitata_ five, seven and nine weeks after germination.

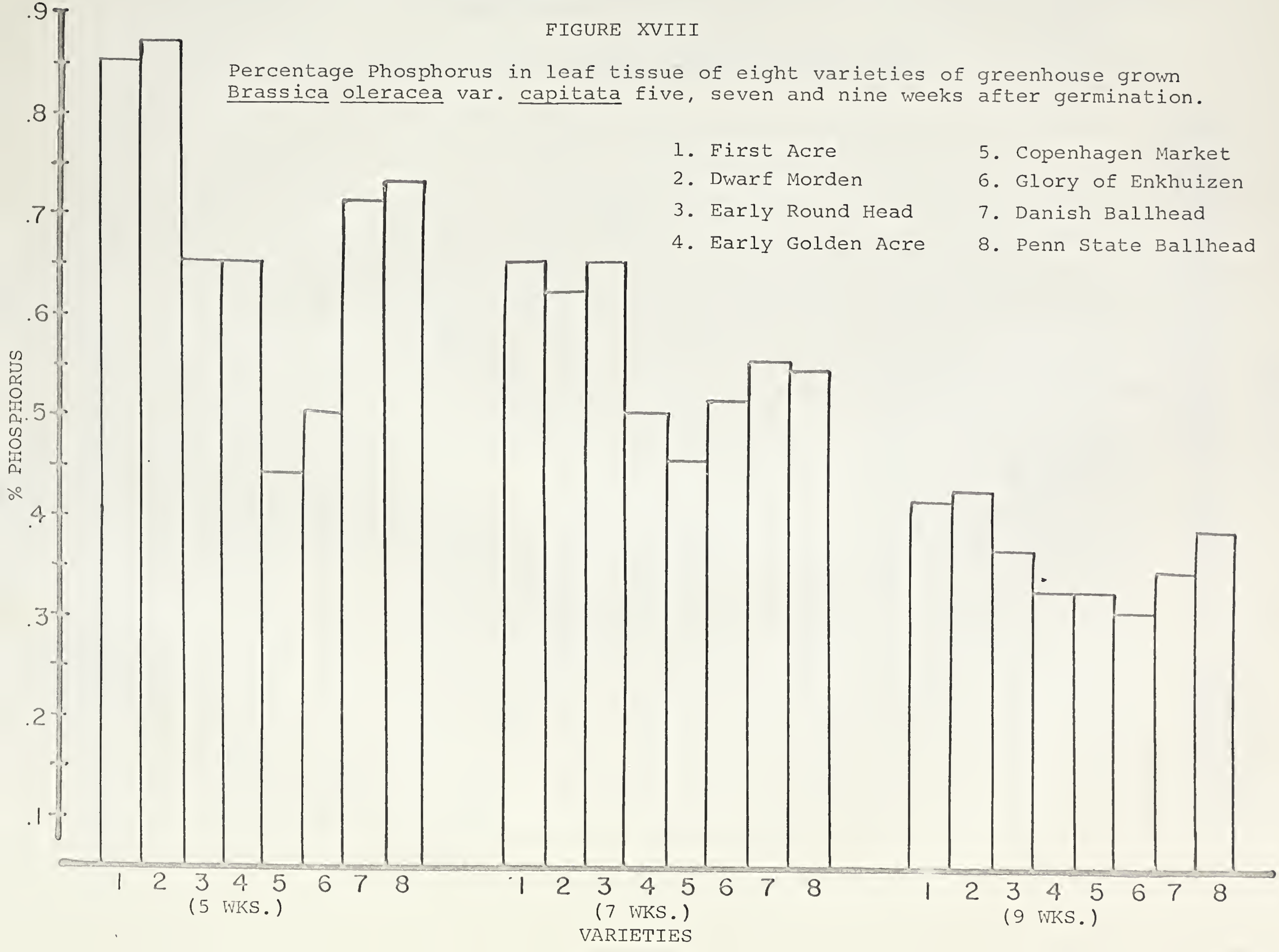
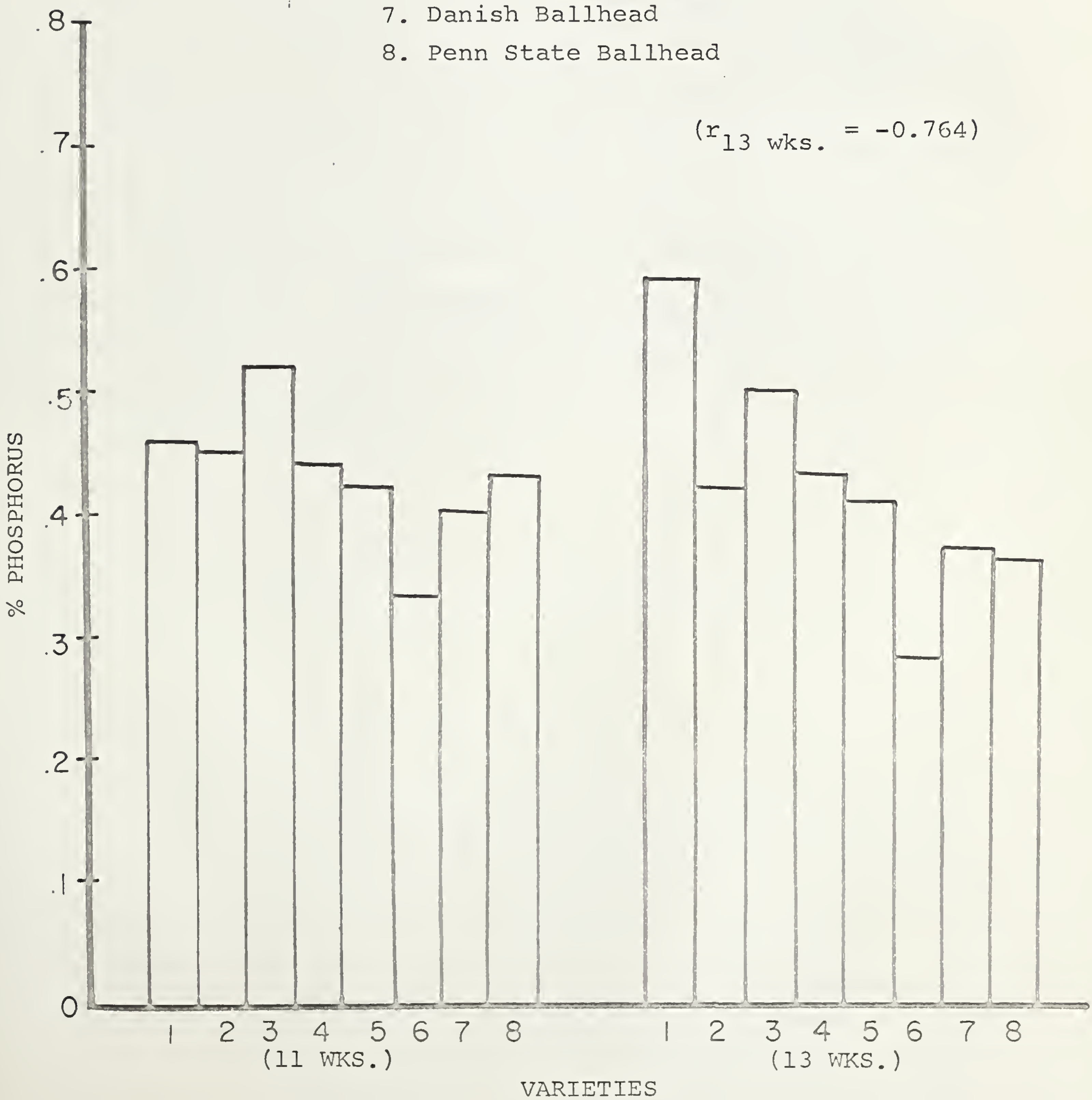


FIGURE XIX

Percentage Phosphorus in leaf tissue of eight varieties of greenhouse grown Brassica oleracea var. capitata 11 and 13 weeks after germination.

1. First Acre
2. Dwarf Morden
3. Early Round Head
4. Early Golden Acre
5. Copenhagen Market
6. Glory of Enkhuizen
7. Danish Ballhead
8. Penn State Ballhead



IV. Radish

There were no differences in dry matter content among varieties at any of the three stages of growth as shown in Table XII. Early varieties had less phosphorus in leaf tissue than varieties with longer maturity periods. The differences in phosphorus levels between various varieties were significant at the 1% level at all the three stages of growth. Duncan's new multiple range test also indicated differences in phosphorus level in varieties of various maturity groups. The results are summarized in Table XIII. The correlation between phosphorus content of leaf tissue and days to maturity was significant at the 1% level at all the three stages as indicated in Figure XX. The regression of % phosphorus in leaf tissue on days to maturity had the greatest slope at 15 days after germination (Figure XXI).

TABLE XII

Days to maturity of eight varieties of <u>Raphanus</u> <u>sativus</u> L. and average % dry matter in leaf tissue at 15, 25 and 35 days after germination

Sample Number	Variety	Days from Germination to Maturity	% Dry Matter		
			15 Days	25 Days	35 Days
1	Cavalier	35	7.83	7.00	6.78
2	Champion	37	7.32	6.94	7.07
3	Forcing Scarlet Globe	40	7.75	6.59	6.62
4	Sparkler	42	7.19	6.21	6.45
5	Long White Icicle	50	7.28	6.41	6.05
6	White Strasburg	59	7.85	6.40	6.27
7	Chinese Rose	68	7.12	6.30	6.50
8	Long Black Spanish	74	8.16	6.63	6.88

TABLE XIII

Days to maturity of eight varieties of *Raphanus sativus* L. and average % phosphorus content in dry leaf tissue at 15, 25, and 35 days after germination

Sample Number	Variety	Days from Germination to Maturity	% Phosphorus 15 Days	% Phosphorus 25 Days	% Phosphorus 35 Days
1	Cavalier	35	1.15[a] [1]	0.58[a] [1]	0.46[a] [1]
2	Champion	37	1.22[a]	0.59[ab]	0.59[bc]
3	Forcing Scarlet Globe	40	1.17[a]	0.56[a]	0.55[b]
4	Sparkler	42	1.22[a]	0.59[ab]	0.60[bcd]
5	Long White Icicle	50	1.29[ab]	0.63[bc]	0.65[cde]
6	White Strasburg	59	1.55[c]	0.66[cd]	0.69[e]
7	Chinese Rose	68	1.45[bc]	0.69[d]	0.67[de]
8	Long Black Spanish	74	1.62[c]	0.68[cd]	0.68[e]
Correlation between % phosphorus in leaf tissue and days to maturity			+0.941**	+0.881**	+0.850**

F values for % phosphorus were significant at 1% level at all the three stages.

**Significant at 1% level.

[1]Numbers in each column which are not followed by the same letter are significantly different from each other at 5% level of significance as judged by Duncan's new multiple range test ( ).

FIGURE XX

Percentage Phosphorus in leaf tissue of eight varieties of greenhouse grown Rapharus sativus 15, 25 and 35 days after germination.

1. Cavalier
2. Champion
3. Forcing Scarlet Globe
4. Sparkler
5. Long White Icicle
6. White Strasburg
7. Chinese Rose
8. Long Black Spanish

($r_{15\ days} = +0.941$) ($r_{25\ days} = +0.881$) ($r_{35\ days} = +0.850$)

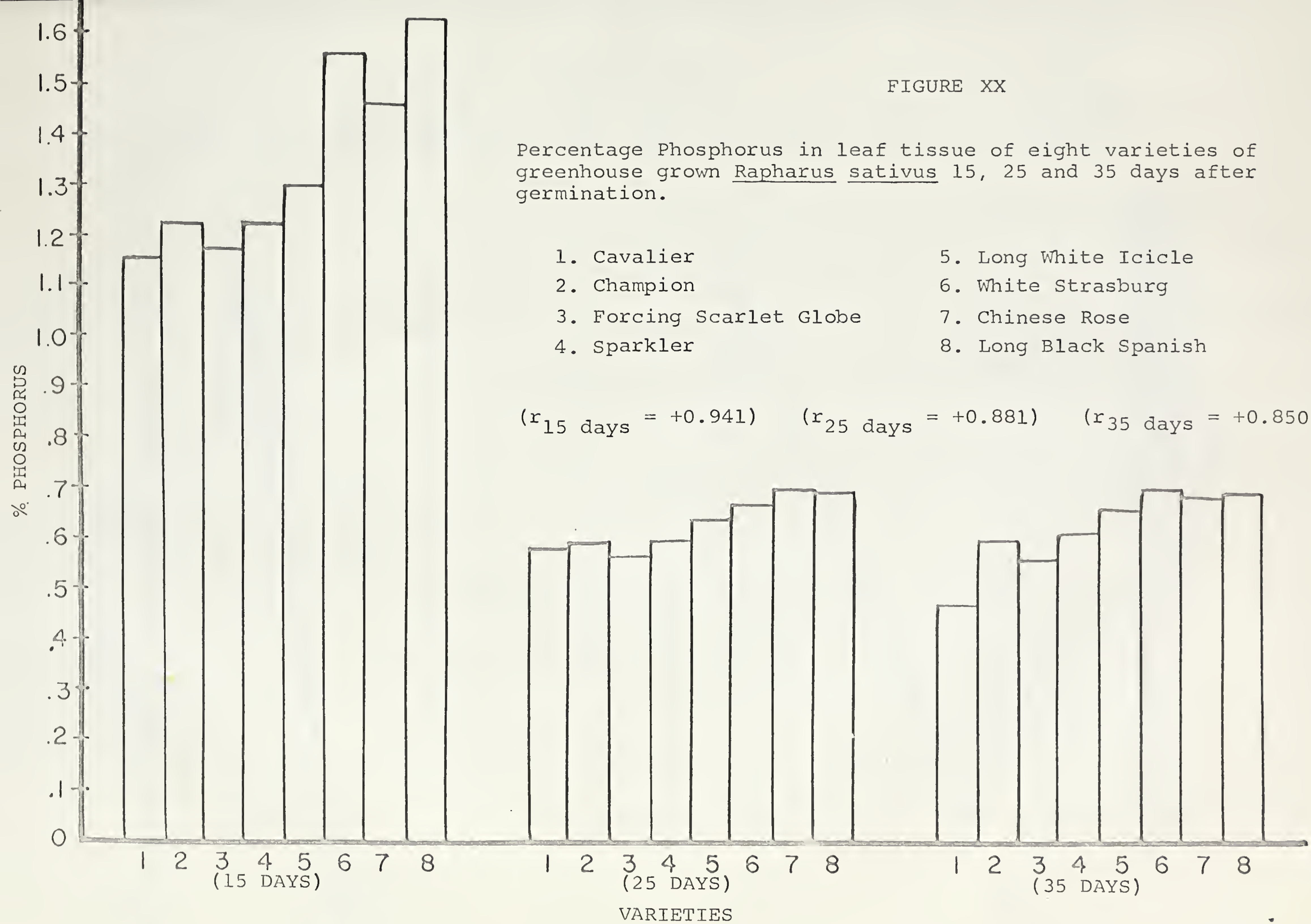
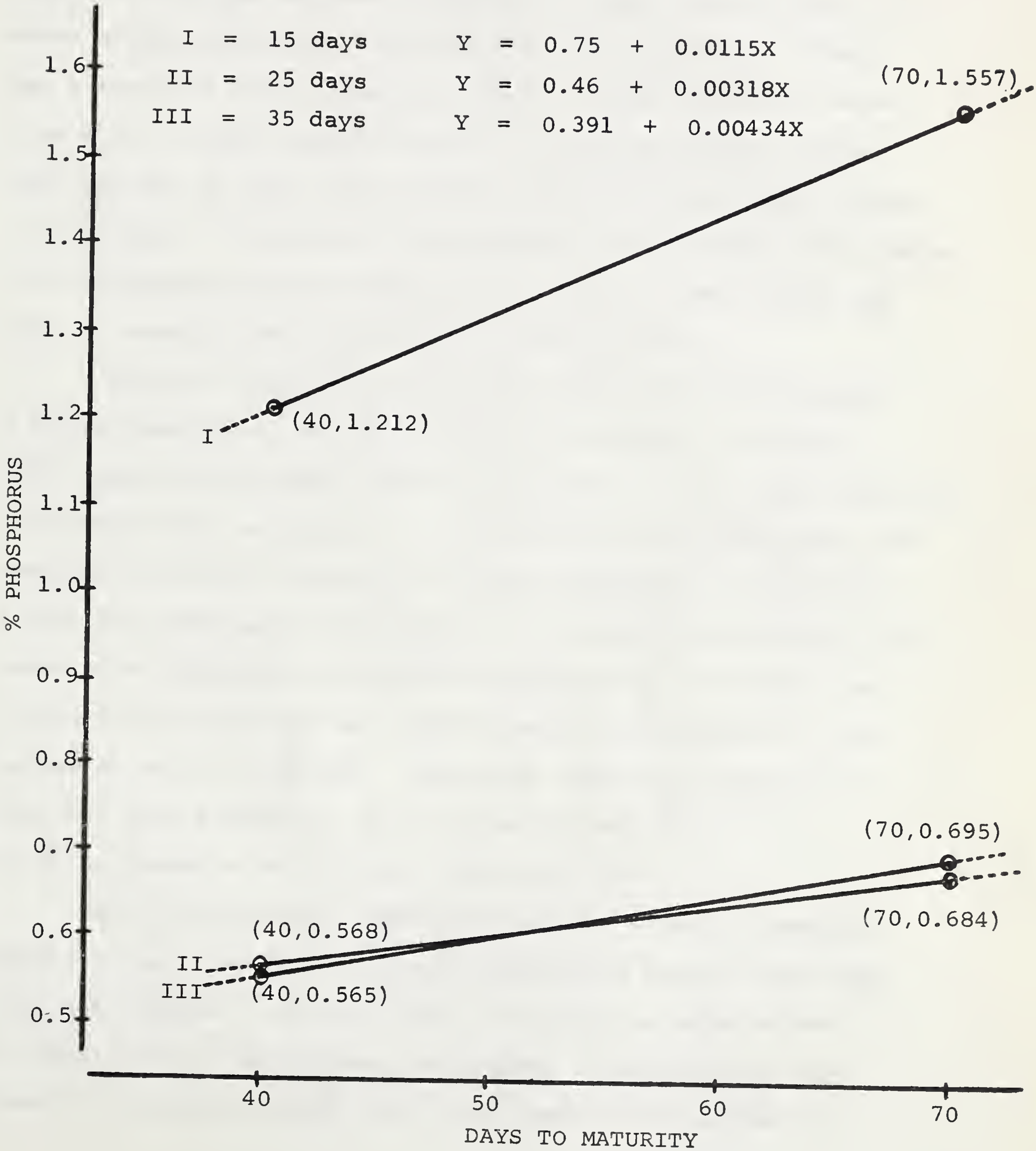


FIGURE XXI

Regression of phosphorus content of leaf tissue of <u>Raphanus sativus</u> L. 15, 25 and 35 days after germination on days to maturity.



DISCUSSION AND CONCLUSIONS

In the first greenhouse experiment with tomatoes, the earlier varieties had more phosphorus in leaf tissue eight weeks after germination than did the later varieties. There was a trend to lower phosphorus level in leaf tissue of varieties with a longer maturity period. Only one variety, Bonny Best was out of line, with slightly more phosphorus than others in its group. In spite of the deviation of the Bonny Best plants the correlation between phosphorus content of leaf tissue and days to maturity was significant at the 1% level.

Since the results of this first test were encouraging a second experiment was initiated in the autumn of 1965 and leaf tissue samples were taken at intervals of two weeks starting six weeks after germination. As in the previous experiment the earliest varieties had about 33% more phosphorus in their leaf tissue six weeks after germination and 20% more phosphorus eight weeks after germination respectively than did the latest ones. The earliest varieties had significantly more phosphorus than medium maturing varieties. The medium maturing varieties in turn had more phosphorus than the latest maturing one as shown by Duncan's new multiple range test (45)

One late variety, Ponderosa deviated from its expected position, as a result of a higher phosphorus content than had been anticipated. Care was taken to supply an equal amount of fertilizer to each plant and samples of each variety were taken in a uniform manner (38, 41). Hence there appears to

be no logical explanation in reference to procedure for the higher phosphorus content of Ponderosa. This variety apparently has more phosphorus in leaf tissue than other varieties in this group. This higher level may be due either to more absorption (41) or differences in translocation (1). With this variety excluded in the calculations the correlation between phosphorus content and days to maturity was significant at the 1% leve. With Ponderosa data included no significant correlation was obtained.

The differences in phosphorus levels between early and late varieties were highest six weeks after germination. These differences became less as the plants advanced in age. Phosphorus content decreased in plant tissue in general as the age of plant increased, which confirms previous results (41).

Ten weeks after germination the phosphorus content in leaf tissue of the earlier varieties fell very rapidly and no correlation was obtained at this stage of growth. This phenomenon appears logical based on the fundamental knowledge that phosphorus has been found to be a mobile nutrient in plants and moves to the organs which are metabolically more active (1). The earlier varieties at this time were flowering and fruiting profusely. There is a high demand for phosphorus in the flowering and fruiting portions of the plant (17). Hence there was a rapid translocation of phosphorus from leaf tissue to flowering parts which resulted in lowering the phosphorus content of leaf tissue in the earlier varieties.

The results were similar at twelve weeks after germination but the reduction in phosphorus level of earlier varieties was more regular than at ten weeks after germination.

The correlation between phosphorus content of leaf tissue and days to maturity was highest six weeks after germination which might be of help as it is useful to have an indication of earliness very early in plant growth. The regression line had greater slope at the six week stage than at the eight week stage indicating greater changes in percentage phosphorus with days to maturity at the six week stage.

Results with the lettuce crop were similar to those with tomatoes. In all three experiments, one in the field laboratory and two in the greenhouse, earlier varieties had more phosphorus in the leaf tissue than did later varieties. In the field plots, nine weeks after germination, there was a negative correlation of 0.75 between % phosphorus in leaf tissue and days to maturity. In the spring experiments in the greenhouse the earliest variety had about 45% more phosphorus than the latest variety. Duncan's new multiple range test showed significant differences in phosphorus content between varieties of different maturity groups.

In a third experiment conducted during the autumn of 1965, the results were similar at all the four stages of growth. Differences of up to 87.6% in phosphorus content of leaf tissue were correlated with days to maturity.

In lettuce, as in tomatoes, the phosphorus content of leaf tissue decreased as the plants advanced in age. This confirms previous work by Smith (41). The differences in phosphorus levels between early and late varieties also became less pronounced with increase in age of the plants. The phosphorus level at four weeks ranged from 1.20 to 0.85% and at ten weeks from 0.75 to 0.54%. In explanation, we would state that the phosphorus supply in the soil at early stages of plant growth was much greater than in the later stages. Phosphorus fertilizer was applied at frequent intervals in the early stages of plant growth and phosphorus content of plant tissue is directly proportional to the supply of nutrients (17, 38). Moreover, the accumulation of carbohydrates as the plants become older dilutes phosphorus concentration (8).

The correlation between phosphorus content of leaf tissue and days to maturity increased from the fourth week after germination to eighth week and then started to decrease again although it was significant at all stages of growth. The slope of the regression line was greatest at six weeks after germination which indicated greater differences in phosphorus level with variable maturity period at this stage.

In cabbage the dry weight of leaf tissue was greater in late varieties than in early ones at ten weeks after germination. The fluctuation of phosphorus content in leaf tissue in cabbage was the reverse of the change which occurred in tomato and lettuce plants. Unlike the previous two crops

earlier varieties had less phosphorus in leaf tissue than the later ones. The correlation between phosphorus in leaf tissue and days to maturity was significant at the 1% level. Although differences in percentage phosphorus between earlier and later varieties were slight, the latest varieties had about 24% more phosphorus in leaf tissue than did the earliest varieties. Lesser differences were to be expected in samples taken at a later stage because the concentration of phosphorus in the leaf tissue decreases with age of the plant (41).

The results with the radish crop were similar to those with cabbage. In the field laboratory tests there were no differences in dry weight content among various varieties but five weeks after germination later varieties had about 45% more phosphorus in their leaf tissue than did the earlier varieties.

In another greenhouse experiment conducted during the autumn of 1965 the results were similar to the above experiment. Fifteen days after germination later varieties had about 45% more phosphorus in leaf tissue than did earlier ones. Twenty-five and 35 days after germination the results were similar but differences in phosphorus levels between earlier and later varieties were much less. There was a general reduction in phosphorus level in all varieties at the second stage of growth which confirm previous findings (17, 41).

The correlation between phosphorus level and days to maturity was significant at the 1% level in all the three stages of growth. However, the highest correlation was found 15 days after germination. Differences of up to 88.5% in

phosphorus content of leaf tissue were correlated with days to maturity. The regression line also had the steepest slope 15 days after germination.

It is apparent from the results of present investigations that in the crops studied, phosphorus content in leaf tissue is correlated with earliness. In tomatoes and lettuce there is a negative correlation between phosphorus level and days to maturity. In cabbage and radish there is a positive correlation.

At this time there is no evidence as to why there is a negative correlation in the first two crops and a positive correlation in the latter two. The negative correlation might be explained by the fact that a higher phosphorus level in plant tissue might help in hastening physiological processes and there is evidence to support that point of view (8, 24, 36, 42, 43). If that point of view holds there can be no explanation for the positive correlation. Moreover, it is interesting to note that both the crops which had positive correlation of phosphorus content with days to maturity belong to the same family. It may be that a genetic character in these species is responsible for such a phenomenon. This might be true for Cruciferea family. However, further investigations are necessary before any definite explanation can be advanced. Many other reasons for this behaviour may be determined by further investigations.

Whatever may be the explanation for these results, the correlation may help in providing some indication of earliness in varieties of these four vegetable crops without growing them to the marketable stage.

Moreover, a correlation established between phosphorus content and earliness might enable breeding for earliness to be undertaken in a different way. For example the genetics of phosphorus absorption might be investigated to bring about a pooling of all the genes contributing to this phenomenon thus enabling more effective selection for early maturity.

PART TWO

THE RELATIONSHIP BETWEEN CHLOROPHYLL (a + b) CONTENT OF LEAF TISSUE AND EARLINESS AT VARIOUS GROWTH STAGES OF THREE VEGETABLE CROPS

MATERIALS AND METHODS

The leaf tissue samples taken at various stages of growth in tomato, lettuce and cabbage, as described under phosphorus determination, were also used for chlorophyll (a + b) determinations.

The process consisted of weighing accurately 50 to 70 mg of dried, ground and homogenized leaf tissue from each of the samples and extracting the chlorophyll with 80% reagent grade acetone. Eighty percent rather than 100% acetone was used for the extraction of dried samples as no water was present in the tissue. A small amount of calcium carbonate was added to the sample before extraction. The extract was filtered through a Whatman No. 1 filter paper into a 50 ml volumetric flask, washed with further aliquots of acetone and total volume made up to the 50 ml mark. The flask was then shaken well for thorough mixing.

Soon after extraction three ml aliquots of chlorophyll extract from each sample were placed in a 1 cm square quartz cuvette and the absorbances determined at wave lengths of 645 and 663 mμ, on a Beckman DK-1 recording spectrophotometer. Eightly percent acetone was used as a standard for comparison. The absorbance readings from the three determinations were

averaged to give the absorbance value for each treatment sample. The average absorbance values were converted to mg chlorophyll per gram dry weight by the use of the following conversion formula of MacLlachlan and Zalik (26).

$$C\,a = \frac{(0.0123 \times D_{663} - 0.00086 \times D_{645}}{W \times d} \times V$$

$$C\,b = \frac{(0.0193 \times D_{645} - 0.0036 \times D_{663}}{W \times d} \times V$$

where C = concentration in mg/gm dry weight; a = chlorophyll a; b = chlorophyll b; D = Absorbance at the wavelength indicated; V = total volume of extract in ml (50 ml); d = length of light path in cms (1 cm); and W = Weight of dry material in gm.

RESULTS

I. Tomatoes

Six weeks after germination total chlorophyll content in later varieties was higher than in earlier varieties as shown in Table XIV and Figure XXII. At this stage of growth the correlation between chlorophyll content of leaf tissue and days to maturity was significant at the 1% level. The slope of regression of chlorophyll content on days to maturity was 0.0233 as seen in Figure XXIII.

Eight and ten weeks after germination there were no significant correlations between chlorophyll content of leaf tissue and days to maturity.

TABLE XIV

Days to maturity of eleven varieties of *Lycopersicon esculentum* L. and chlorophyll (a + b) content of dry leaf tissue at six, eight and ten weeks after germination

Sample Number	Variety	Days from Germination to Maturity of First 3 Fruits	Chlorophyll (a + b) in mg/gm. 6 Weeks	8 Weeks	10 Weeks
1	B.V. 5	96	4.203	8.346	10.356
2	Rocket	97	5.207	8.055	12.773
3	Earlinorth	100	5.455	8.278	13.359
4	Johnny Jumpup	100	5.882	6.937	12.910
5	Early Lethbridge	107	6.350	8.623	8.931
6	Manitoba	116	6.645	7.306	8.857
7	Bonny Best	118	6.270	8.817	10.237
8	Crimson Cushion	130	6.617	6.092	9.863
9	Beefsteak	138	6.977	7.978	10.518
10	Ponderosa	144	6.705	7.549	9.982
11	Pearson	147	6.798	6.597	12.025
Correlation between chlorophyll (a + b) content in leaf tissue and days to maturity			+0.762**	N.S.	N.S.

**Significant at 1% level.

FIGURE XXII

Chlorophyll (a + b) content of leaf tissue of eleven varieties of greenhouse grown <u>Lycopersicon esculentum</u> six weeks after germination.

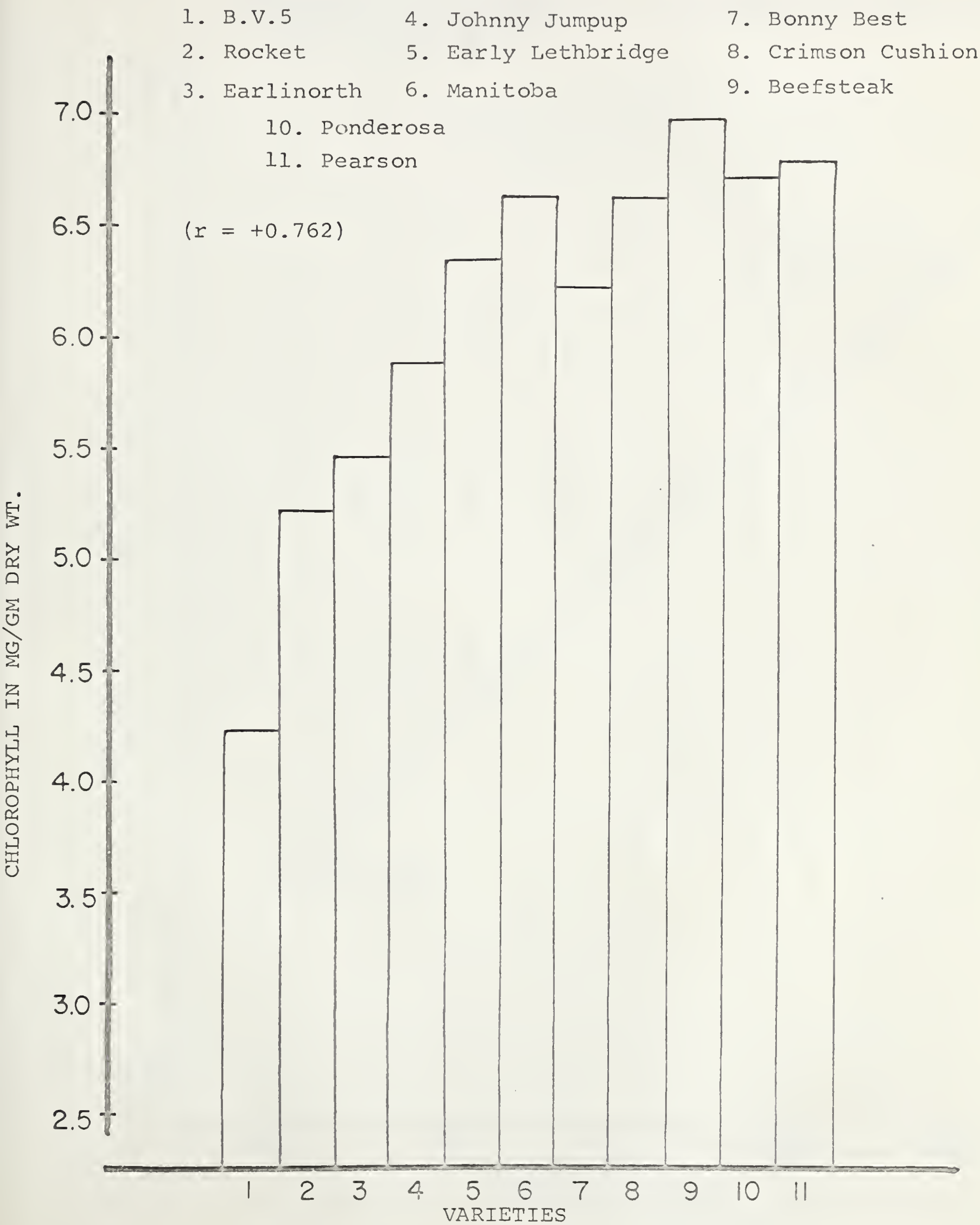
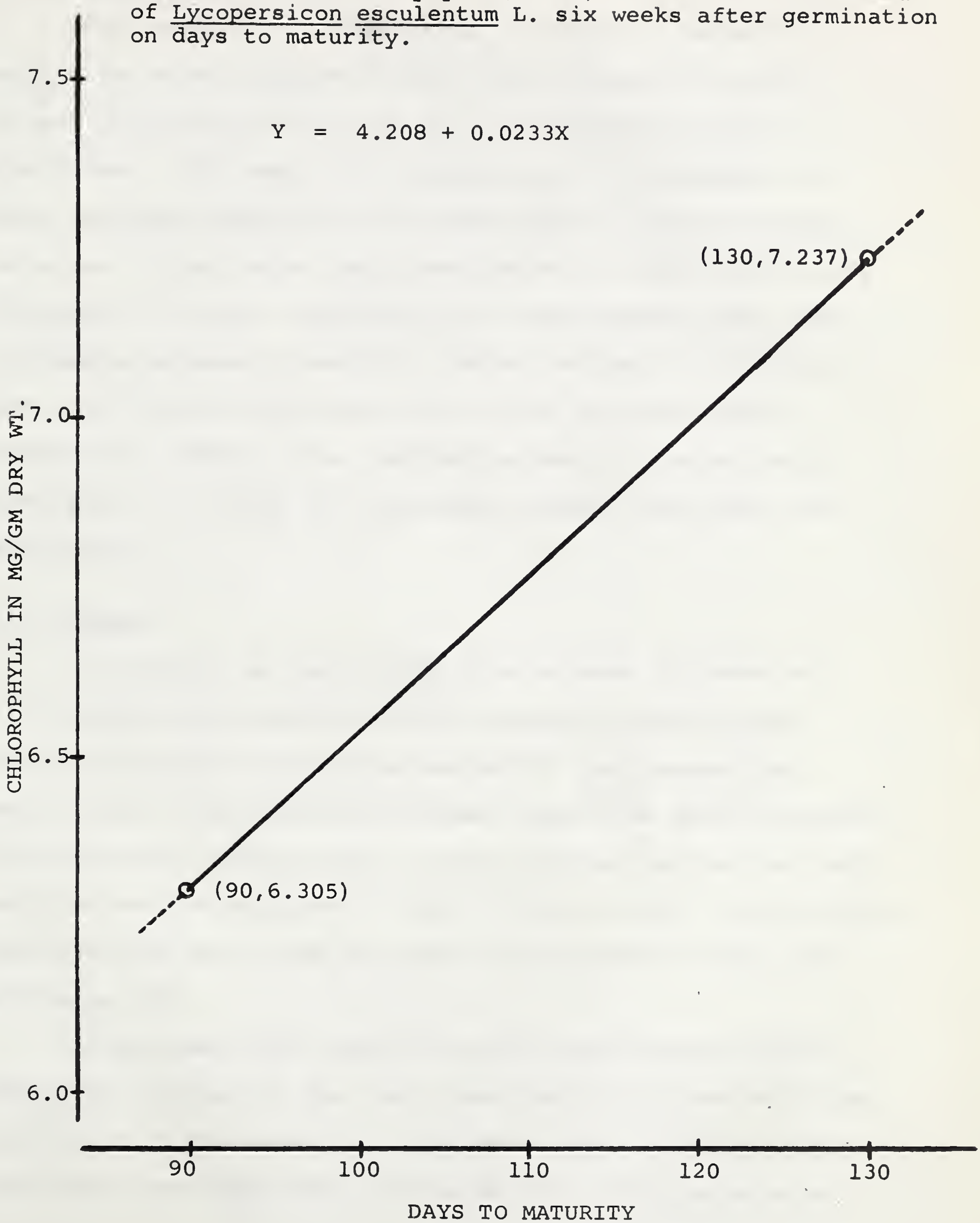


FIGURE XXIII

Regression of chlorophyll (a + b) content of leaf tissue of Lycopersicon esculentum L. six weeks after germination on days to maturity.



II. Lettuce

Four weeks after germination, as shown in Table XV, earlier varieties appeared to have more chlorophyll content of leaf tissue than later ones but the differences were not significant. Six weeks after germination the chlorophyll in later varieties appeared to be almost equal to that of earlier varieties. Eight weeks after germination the trend was to more chlorophyll in later varieties than in the earlier ones. The correlation between chlorophyll content and days to maturity eight weeks after germination was -0.789, as summarized in Figure XXIV. However, the correlation was not statistically significant. F values for chlorophyll content also were non-significant.

III. Cabbage

In cabbage, at each of the three stages of growth at which samples were analysed for chlorophyll content, later varieties had more chlorophyll content of leaf tissue than earlier ones. The differences became greater as growth advanced. The differences in chlorophyll content between earlier and later varieties were significant at the 1% level at both the seven week stage and nine week stage of growth as presented in Table XVI and Figure XXV.

Seven weeks after germination the correlation between chlorophyll content of the leaf tissue and days to maturity was significant at the 5% level. Nine weeks after germination the correlation was significant at the 1% level. The correlation

TABLE XV

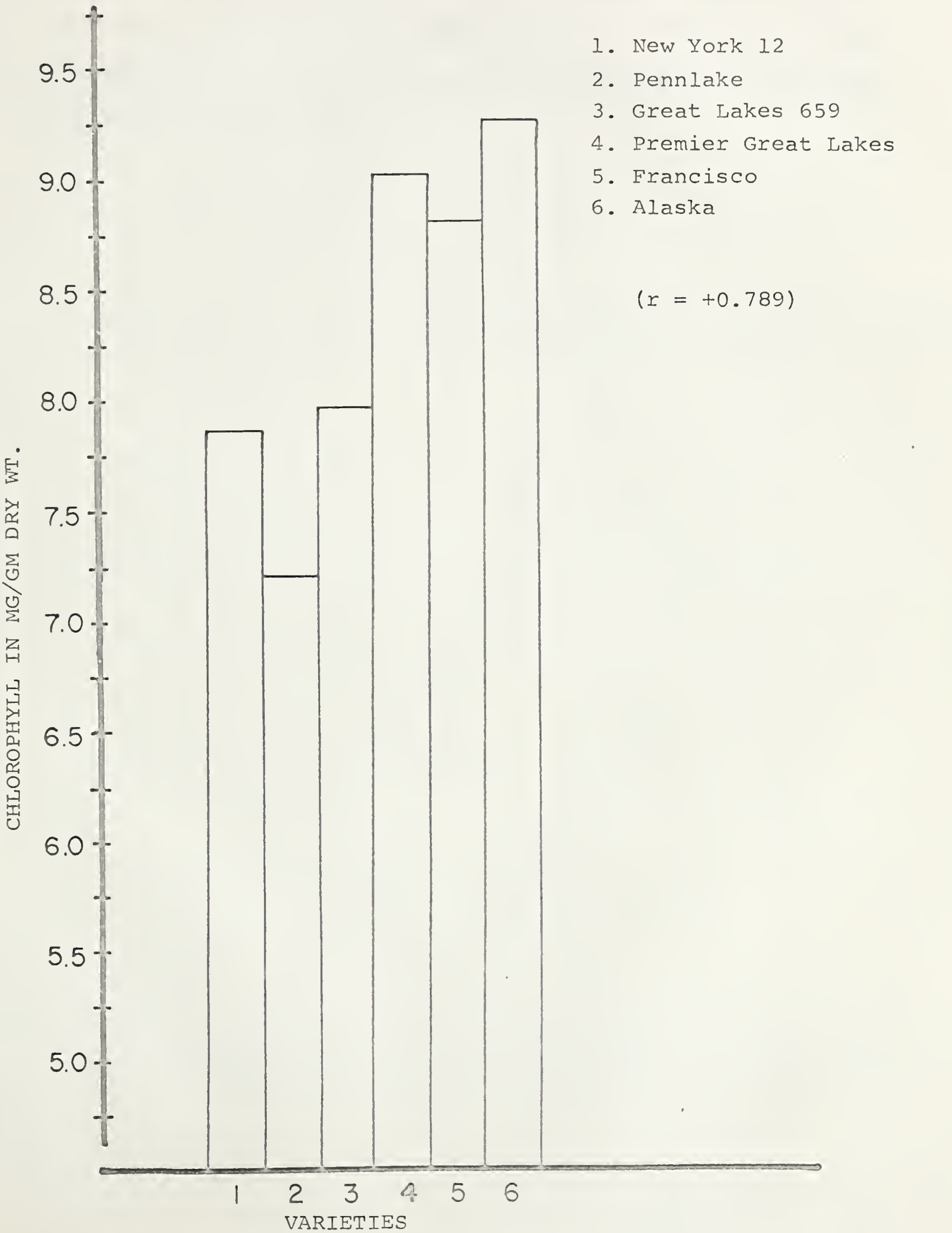
Days to maturity of six varieties of Lactuca sativa. var. capitata L. and chlorophyll (a + b) content of dry leaf tissue at four, six, and eight weeks after germination

Sample Number	Variety	Days from Germination to 60% Marketable Heads	Chlorophyll (a + b) in mg/gm. 4 Weeks	6 Weeks	8 Weeks
1	New York 12	77	9.284	6.789	7.865
2	Pennlake	82	9.711	5.745	7.210
3	Great Lakes 659	83	9.206	6.773	7.954
4	Premier Great Lakes	86	8.456	6.877	9.019
5	Francisco	92	9.385	6.863	8.815
6	Alaska	95	8.323	6.453	9.255
Correlation between chlorophyll (a + b) content in leaf tissue and days to maturity			-0.572 N.S.	+0.099 N.S.	+0.789 N.S.

F values for chlorophyll (a + b) content were not significant.

FIGURE XXIV

Chlorophyll (a + b) content of leaf tissue of six varieties of greenhouse grown Lactuca sativa var. capitata eight weeks after germination.



at the five week stage was not significant. As can be seen in Figure XXVI the slope of regression of chlorophyll on days to maturity at nine weeks was 0.0312 compared to 0.292 seven weeks after germination.

TABLE XVI

Days to maturity of eight varieties of *Brassica oleracea* var. *capitata* L. and chlorophyll (a + b) content of dry tissue at five, seven and nine weeks after germination

Sample Number	Variety	Days from Germination to 70% Marketable Heads	Chlorophyll (a + b) in mg/gm. 5 Weeks	7 Weeks	9 Weeks
1	First Acre	96	5.970	5.416[a] [1]	5.700[a] [1]
2	Dwarf Morden	98	6.294	5.551[ab]	6.068[abc]
3	Early Round Head	98	5.583	6.690[c]	5.901[ab]
4	Early Golden Acre	111	5.726	7.138[c]	6.808[cd]
5	Copenhagen Market	115	6.281	6.410[bc]	6.594[bc]
6	Glory of Enkhuizen	135	6.334	7.212[c]	6.788[cd]
7	Danish Ballhead	148	6.815	7.405[c]	7.510[de]
8	Penn State Ballhead	152	6.772	7.543[c]	7.814[e]
Correlation between chlorophyll (a + b) content in leaf tissue and days to maturity			+0.438 N.S.	+0.823*	+0.947**

F values for chlorophyll (a + b) content were significant at 1% level at seven and nine weeks after germination.

*Significant at 5% level.

**Significant at 1% level.

[1]Numbers in each column which are not followed by the same letter are significantly different from each other at 5% level of significance as judged by Duncan's new multiple range test ( ).

FIGURE XXV

Chlorophyll (a + b) content of leaf tissue of eight varieties of greenhouse grown <u>Brassica</u> <u>oleracea</u> var. <u>capitata</u> seven and nine weeks after germination.

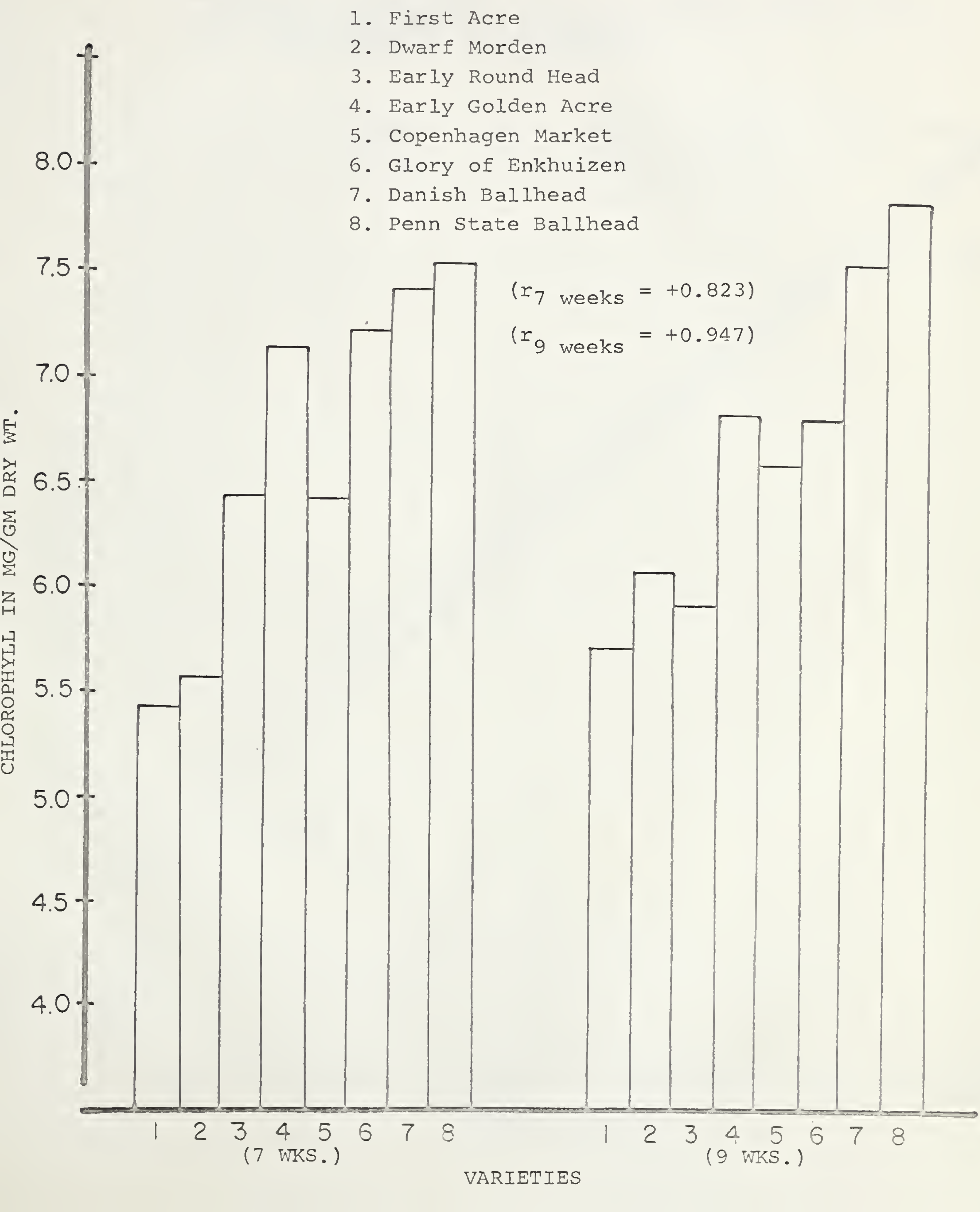
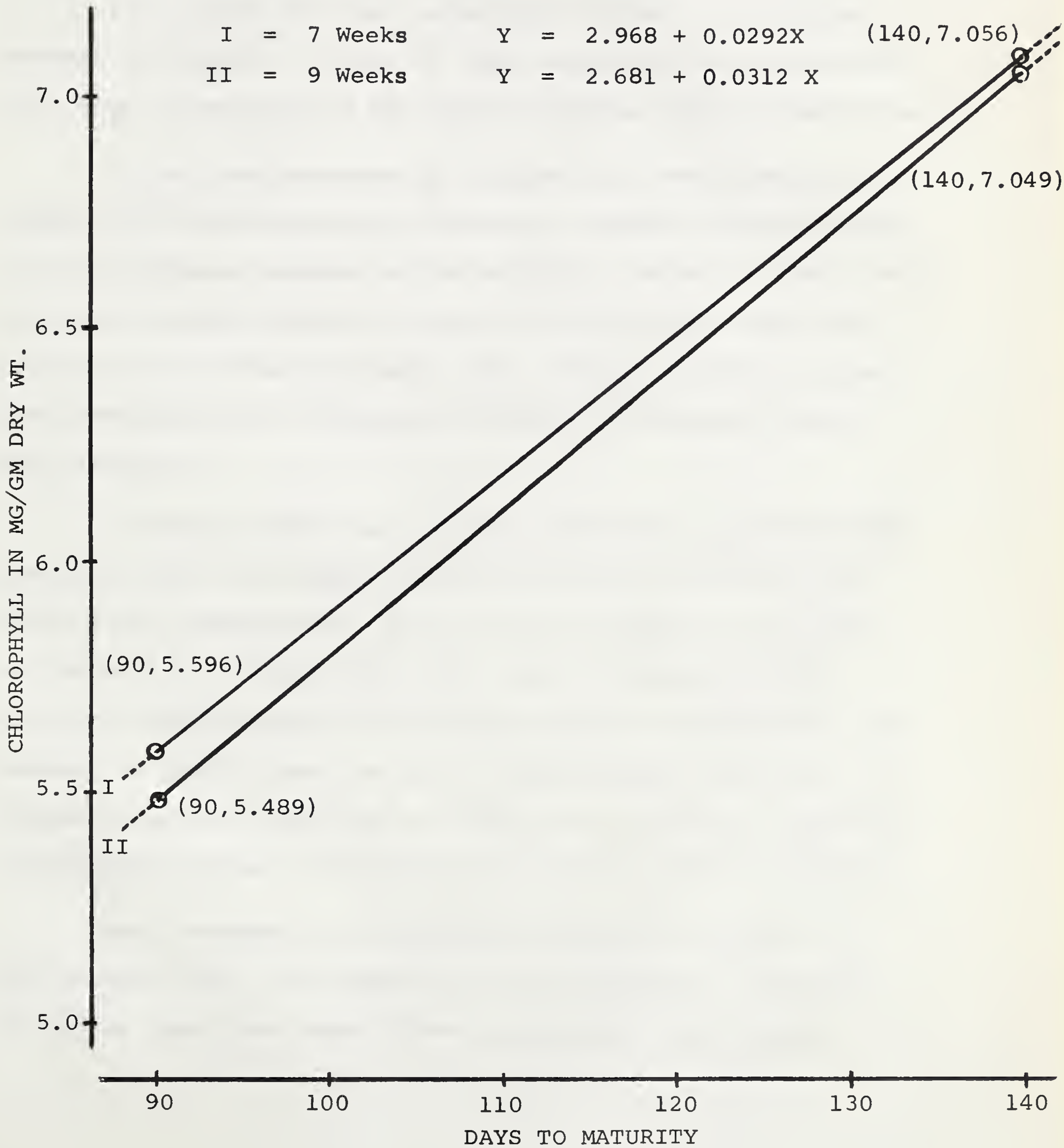


FIGURE XXVI

Regression of chlorophyll (a + b) content of leaf tissue of <u>Brassica</u> <u>oleracea</u> var. <u>capitata</u> L. seven and nine weeks after germination on days to maturity.



DISCUSSION AND CONCLUSIONS

The purpose of these investigations was to determine whether chlorophyll content in leaf tissue had any correlation with days to maturity of the three vegetable crops investigated.

It has been reported by Brougham (6) that there is a highly significant positive correlation between maximum growth rate of different species and chlorophyll content in leaf tissue. Generally earlier plants in most of the vegetable crops are smaller in size than the later ones. These two facts suggest the possibility of a correlation between chlorophyll content and maturity.

In tomato there was a highly significant positive correlation between chlorophyll content and days to maturity six weeks after germination. This one might expect on the basis of the work by Brougham (6). The early varieties of this crop are usually smaller determinate types of plants with less vegetative growth than the later indeterminate varieties. At stages of growth later than six weeks there were no significant correlations between chlorophyll content and days to maturity.

Very interesting resulted were noted in the case of the lettuce crop. As evident from the chlorophyll analysis of leaves taken four weeks after germination, the earlier

varieties appeared to develop chlorophyll more rapidly at an earlier date than did later varieties. As growth and development progressed the chlorophyll content of later varieties almost equalled that of earlier ones. In the final stages of vegetative growth, samples of later varieties appeared to contain more chlorophyll than earlier ones.

In cabbage earlier varieties had less chlorophyll content the leaf tissue than later ones at each of the three stages of growth at which chlorophyll was tested. Earlier varieties of cabbage developed a greater proportion of their chlorophyll content at an earlier date than did later varieties. Earlier varieties contained almost the same amount of chlorophyll at all three stages whereas the chlorophyll content of the later varieties increased with the age of the plant from five weeks to nine weeks after germination. This can also be seen in the correlation coefficients and slope of regression lines (Table XVI and Figure XXVI). Five weeks after germination there was no significant correlation between chlorophyll content and days to maturity, seven weeks after germination a positive correlation was significant at the 5% level and at nine weeks the correlation was significant at the 1% level. Also, the slope of the regression line at nine weeks was steeper than the slope of line representing the regression at seven weeks after germination.

The higher correlation at a later stage of growth appears logical on the basis of previous work (5, 6). The

differences in size of plants between early and late varieties are not pronounced at the earlier stages of growth. The differences in size became more apparent as the plants advanced in age.

The genetic influence on chlorophyll content of leaf tissue (18), suggests that genes responsible for early maturity might be linked with genes which control the production of chlorophyll in the leaf tissue. The lower chlorophyll level in leaf tissue of earlier varieties of tomato and lettuce could also be associated with more phosphorus (11, 12, 35); which in turn is associated with less nitrogen (7, 21, 23, 43) in the leaf tissue. As discussed in the previous section of this thesis the earlier varieties of tomatoes and lettuce had more phosphorus than later varieties and hence nitrogen may have been a limiting factor (43). The limited amount of nitrogen is, at least, partially responsible for less chlorophyll synthesis in earlier varieties (51). In cabbage we cannot justify the relationship of chlorophyll content with phosphorus. It may be attributable to genetic factor or factors operative only in this crop.

In conclusion we may mention that in each of the three crops tested for chlorophyll content there was a positive correlation between days to maturity and chlorophyll content of leaf tissue at some stage of plant growth. Hence this positive correlation between chlorophyll content and days to maturity could also be utilized in obtaining some indication of early maturity at early stages of plant growth. If a correlation could be established at a particular stage of growth we could study the

genetic relationship to chlorophyll content in leaf tissue and try to use this character indirectly to assist in breeding for earliness.

PART THREE

THE RELATIONSHIP BETWEEN ORGANIC ACID CONTENT OF FRUITS AND EARLINESS IN *Lycopersicon esculentum* L.

MATERIALS AND METHODS

Tomato fruits from the greenhouse experiments, conducted during the spring of 1965, described in Part One of this thesis, were used for the estimation of organic acids. The organic acid estimations were made at three stages of fruit development:

(1) Mature green stage,

(2) Early ripe stage,

(3) Late ripe stage.

The juice from the fruits was extracted immediately after harvest. It was cleared from interfering substances by the method of Ranson (37). Ten ml of filtrate was combined with 14 gm of washed Celite. The mixture was slurried with butanol-chloroform (50:50) solvent and transferred quantitatively to a chromatography tube. The organic acids were extracted by further addition of small aliquots of butanol-chloroform (50:50) to the column until the elution was complete. The eluted extract was evaporated in vacuo to a 10 ml volume at a temperature, not exceeding 35° C. This reduced extract was used for qualitative and quantitative estimation.

The qualitative and quantitative estimations were done by paper chromatography using the ascending technique. The ascending technique was preferred over the descending one because the spots were more uniform and better defined. Many

solvents were tried but the best results were obtained with chloroform:95% ethyl alcohol in 1:1 ratio in which 1% formic acid of 90% strength was added (44). To the solvent was added sodium formate 0.05% W/V and bromophenol blue 0.02% W/V for development of spots as reported by Blundstone (3). This method of developing spots was preferred over spraying after drying the paper. The reason for the preference was that regardless of the precautions taken in uniformly spraying the developing material no uniform color background would develop. Moreover bromophenol blue has a very high Rf value which shows up all the organic acids (3). Ten μl of the cleared fruit juice extract solution per spot were applied 10 cm from the bottom with 5 cm horizontal spacing between the spots on Whatman No. 1 chromatographic paper sheets. After the solvent had travelled more than 2/3 of the length of the tank, the sheets were removed and dried overnight in a fume chamber. A small trace of ammonia was added to the atmosphere for distinct development of the spots. Upon drying the acids appeared as yellow spots against a blue background.

The various spots were identified by developing chromatographic sheets with known acids and comparing their spot positions with unknown spots using more than two solvent systems.

Quantitative determinations were made by the spot area method of Bryant and Overall (1953) described by Ranson (37). This method was selected as a substitute for Densicord method because the reading of intensity of acid spots by Densicord did not work properly although all the available

filters at various response numbers were tried. Since the differences in malic acid content between various varieties were large, this method gave a fairly accurate indication of relative amounts of the acids in varieties of different maturity groups. A planimeter was used in measuring the area of the acid spots. The average area of four spots with two measurements for each spot was taken as the area of the malic acid spot for each variety.

A standard curve was obtained by plotting the area of malic acid spots against the log of known amounts of malic acid spotted on the chromatographic sheets.

The area of malic acid spots of unknown amounts from tomato fruit juice of various varieties, spotted on chromatographic paper, were compared with standard curve and the amount in each spot was estimated.

Later the difficulties with the Densicord system were overcome and it was used for quantitative estimation of malic acid concentration.

In the Densicord system the transmission of light is proportional to the density of the acid spot. It is considered to be more accurate than the spot area method previously described.

A standard curve was obtained by plotting area of curve against known amounts of malic acid spotted on the chromatographic sheets.

The area of curve from malic acid of fruit juice of different varieties was compared with the standard curve and amount in each spot estimated.

RESULTS

No relationship was obtained between citric acid content of fruit juice and days to maturity although the amount of citric acid was higher than any other organic acid in tomato fruit juice.

As shown in Tables XVII and XVIII, malic acid content of fruit juice in earlier varieties was higher than in later varieties at all the three stages of fruit ripening. At the mature green stage the correlation between malic acid content of fruit juice and days to maturity was significant at the 5% level by spot area method but at the 1% level by Densicord method. At early ripe stage of fruit ripening the correlation was significiant at the 1% level in both cases. At late ripe stage correlation between malic acid content of fruit juice and days to maturity was significant at the 1% level by area method and at the 5% level by density method. The slope of regression of malic acid content of fruit juice on days to maturity was the highest at mature green stage followed by early and late ripe stages respectively. The regression lines at the three stages of fruit ripening are presented in Figures XXVII and XXVIII.

In all varieties there was a higher level of malic acid in fruit juice at the mature green stage than at early or late-ripe stages.

TABLE XVII

Days to maturity of ten varieties of Lycopersicon esculentum L. and malic acid content of fruit juice at three stages of fruit ripening

Sample Number	Variety	Days from Germination to Maturity of First 3 Fruits	Malic acid in µg/10 µl of fruit juice: Mature Green Stage	Early Ripe Stage	Late Ripe Stage
1	B.V. 5	96	30.20	23.07	12.08
2	Rocket	97	28.18	20.42	10.72
3	Earlinorth	100	32.74	18.24	10.99
4	Johnny Jumpup	100	23.99	20.61	9.93
5	Early Lethbridge	107	21.14	15.74	6.44
6	Alpha #5	113	20.89	15.14	6.43
7	Manitoba	116	18.62	14.03	7.96
8	Bonny Best	118	16.29	12.53	6.41
9	Crimson Cushion	130	22.70	15.85	6.44
10	Beefsteak	138	16.67	10.47	6.38
Correlation between malic acid content of fruit juice and days to maturity			-0.679*	-0.77**	-0.797**

*Significant at 5% level.

**Significant at 1 % level.

TABLE XVIII

Days to maturity of ten varieties of *Lycopersicon esculentum* L. and malic acid content of fruit juice at three stages of fruit ripening (Densicord Method)

Sample Number	Variety	Days from Germination to Maturity of First 3 Fruits	Malic acid in µg/10 µl of fruit juice		
			Mature Green Stage	Early Ripe Stage	Late Ripe Stage
1	B.V. 5	96	44.87	21.68	12.45
2	Rocket	97	40.09	21.18	12.45
3	Earlinorth	100	38.55	20.28	10.64
4	Johnny Jumpup	100	42.07	23.12	11.40
5	Early Lethbridge	107	22.70	19.82	10.89
6	Alpha #5	113	19.41	14.86	10.19
7	Manitoba	116	19.01	15.52	10.19
8	Bonny Best	118	18.16	13.00	9.52
9	Crimson Cushion	130	25.82	16.26	11.67
10	Beefsteak	138	17.74	12.74	9.16
Correlation between malic acid content of fruit juice and days to maturity			-0.782**	-0.851**	-0.645*

*Significant at 5% level.

**Significant at 1% level.

FIGURE XXVII

Regression of malic acid content of fruit juice of Lycopersicon esculentum L. at three stages of fruit ripening on days to maturity.

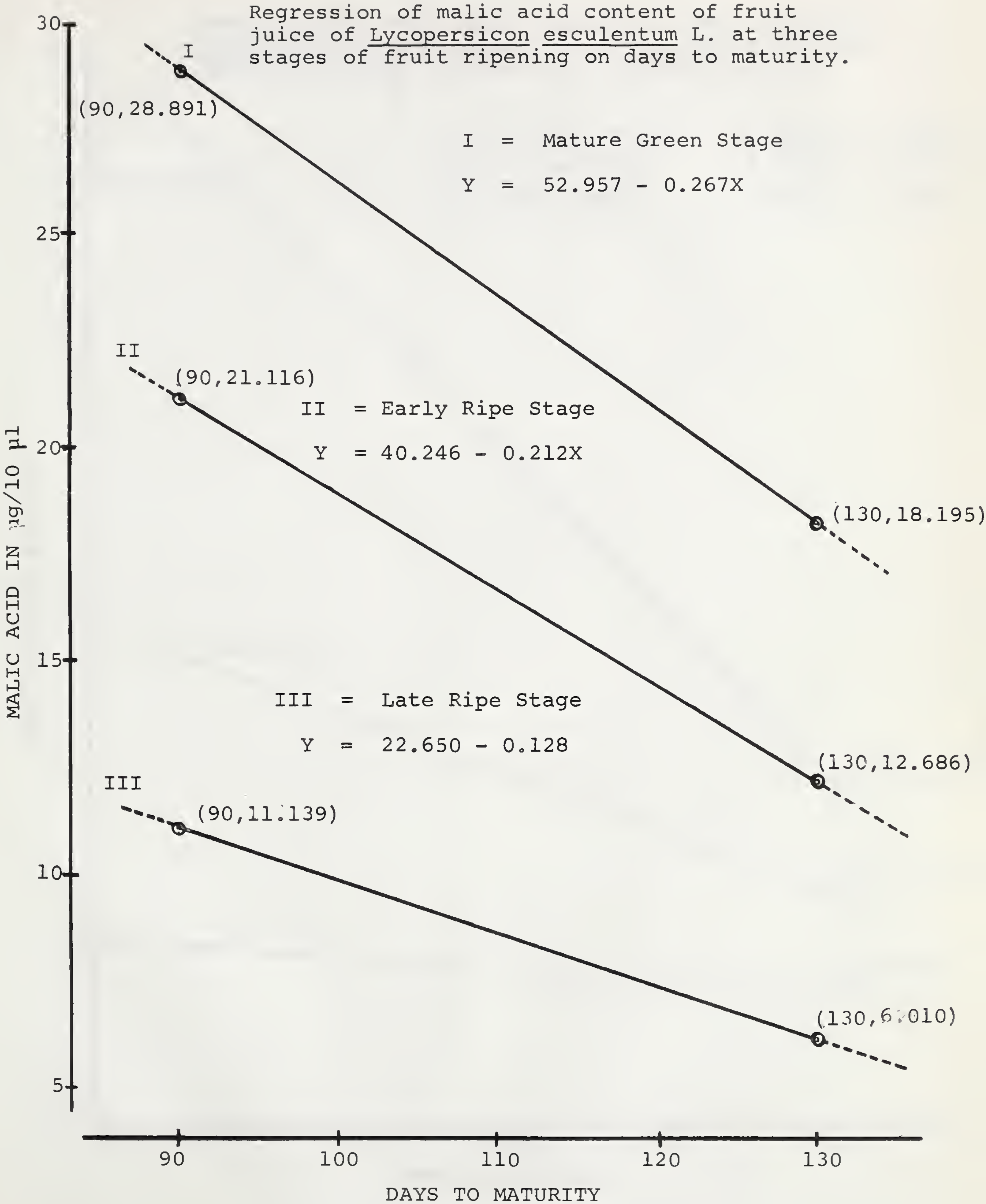
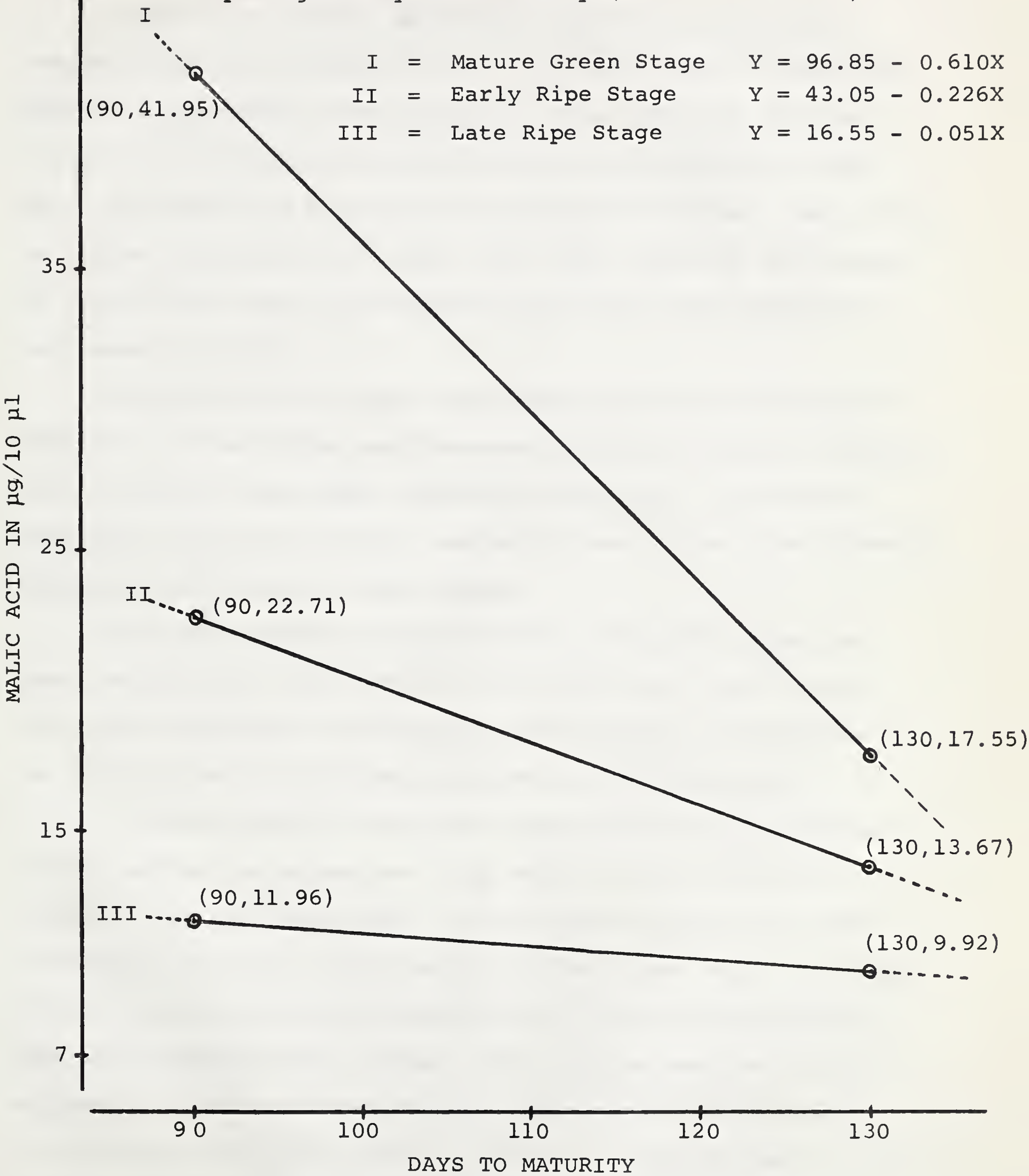


FIGURE XXVIII

Regression of malic acid content of fruit juice of Lycopersicon esculentum L. at three stages of fruit ripening on days to maturity (Densicord Method).



DISCUSSION AND CONCLUSIONS

There was a highly significant negative correlation between malic acid content of fruit juice and days to maturity. This is in agreement with the work of Koch (22) who obtained a significant correlation between malic acid content of fruit juice and days from flowering to ripening of fruits. The earlier varieties generally take lesser time from flowering to ripening of fruits than later varieties and hence the relationship for earliness is logical.

The malic acid content was highest at the mature green stage for all varieties and decreased as fruit ripening advanced. Similar results have been reported by Koch (22). Both malic acid and citric acid content reached a peak in the green ripening stage and decreased in later stages.

One late variety, Crimson Cushion, deviated from its expected position, as a result of a higher malic acid content than other varieties in the late maturing group. This deviation was noted at each of the three stages of fruit ripening.

At the present time we are aware of no explanation as to why earlier varieties have more malic acid in the fruit juice than do the later ones. It is obvious that in the case of earlier varieties, there may be either a more rapid synthesis of malic acid or a slower breakdown of it in the Krebs cycle. This also suggests that enzymes which are responsible for the synthesis and/or breakdown of malic acid may not be produced at the same rate by the earlier varieties as by the later ones. Another possibility may be that some other compound may be

present either in smaller or greater quantities in earlier varieties than in later ones. Either situation could block the forward step from malic acid or accelerate the formation of malic acid in earlier varieties.

On the basis of heridity it appears possible that the genes responsible for production or accumulation of malic acid in tomato fruits are linked with the genes which are responsible for enhancing maturity. It would be interesting to undertake studies to ascertain whether and at what stage in the Krebs cycle the earlier varieties have a differential rate of synthesis or breakdown to account for the differences in malic acid content.

However, whatever may be the reason for higher malic acid content in earlier varieties it can be of great use to vegetable breeders. It can be a laboratory method for testing early maturity which is objective in nature and may be less subject to human error than visual observations.

Breed for earlier ripening varieties might be done by selecting plants with higher malic acid content in their fruit juice. Another method suggested for breeding for early maturity might be the crossing of plants with medium malic acid content but different genetic (factors responsible for malic acid) backgrounds. In the segregating generations there would be a chance of obtaining plants with higher malic acid content than the parental varieties as a result of transgressive segregation. Such methods might prove more effective in breeding for early maturity than those presently in use.

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